Sustainable management of adult *Costelytra zealandica* (Coleoptera: Melolonthinae) damage in Marlborough vineyards

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Mauricio Andrés González-Chang

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Conventional agriculture is facing many challenges to provide food security for a constantly growing human population. Unfortunately, it still relies heavily on fossil fuel-derived inputs, such as pesticides, which affect human health, biodiversity and contribute to global warming. In New Zealand, the endemic grass grub *Costelytra zealandica* (Coleoptera: Melolonthinae) has been a pasture pest for more than 100 years. By consuming plant roots, its larvae reduce pasture yield, affecting both dairy and meat production. Recently, adults of this species have been found feeding on horticultural crops, such as kiwifruit, avocado, and vines, amongst others. In vineyards, adults damage vines by feeding on leaves, shoots and inflorescences and can produce a substantial defoliation, reaching in some cases 100%. For this reason, prophylactic use of synthetic pyrethroids is practised as the main approach to control this pest. Pyrethroids are broad-spectrum insecticides, having clear impacts on arthropod diversity, but also their continuous application can have detrimental effects on human health and, like all such chemicals, can lead to pesticide resistance. Therefore, the aim of this work was to understand *C. zealandica* adult behaviour as it approaches and feeds on vine foliage and thus evaluate a sustainable approach to reduce its damage in vineyards without using pesticides. For this reason, a range of experiments were carried out on vineyards in Marlborough area of New Zealand, in the Awatere Valley (41°44'S; 173°52'E) during 2014 and 2015, and in Blenheim (41°33'S; 173°55'E) during 2015. By studying beetles' distribution, through adult and larval sampling within vineyard blocks, higher adult and larval numbers were found at the edge of the vineyard compared to its centre. This distributional pattern was probably an expression of a relict adult behaviour in this species, in which females respond to plant silhouettes against the sky to feed and mate. Is therefore likely that in Marlborough vineyard-dominated landscapes, vines were the most abundant plant silhouettes, which might explain adult and larval abundance at the margin of studied vineyard blocks. In another experiment, by removing adults that landed on the vine foliage at several times after their daily flight activity started, it was demonstrated that females land before males on the vine foliage. Literature suggests that *C. zealandica* females attract males by releasing

their pheromone, phenol. Based on those results, different experiments to evaluate the contribution of feeding and landing deterrents to reducing beetle damage were established at the studied vineyards. A novel, naturally-based approach to reduce adult damage in Marlborough vineyards by applying silica-derived feeding deterrents, such as hydrophobic particle films (HPF) and diatomaceous earths (DE) on the vine foliage and secondly, by placing crushed mussel shells (MS) (*Perna canaliculus*; Mollusca: Mytilidae) under the vine-row as mulch was investigated in this work. A significant reduction in vine damage was produced by HPF and DE compared to control, with a 46% damage reduction in Pinot Noir variety. MS significantly reduced the number of adults that landed on the vine plants when compared to control in Pinot Noir. To explore potential synergies between HPF and MS, these were combined in another experiment during the 2015 adult flight season at Blenheim. Adult *C. zealandica* damage was significantly reduced by 33 and 73% by HPF and MS, respectively. Using infra-red sensitive video cameras it was demonstrated that MS significantly reduced activity of flying adults above treated vine plants (cv. Pinot Noir), which led to a 28% increase in grape yield. It was suggested that the light reflective properties of MS reduced the necessary plant contrast with the sky, altering adult landing dynamics and subsequently, vine damage. These findings contribute to the reduction of pesticide use within this agro-ecosystem, and also remove the need for disposal of large quantities of these shells, which would otherwise go to the local landfill. There, the proteinaceous parts of the shell waste generate methane, a key contributor to greenhouse gases due to the anaerobic conditions present in the landfill. In addition, this management is in agreement with the cultural perception of kaitiakitanga, which is the spiritual consideration of protecting the land for descendants, rooted in Maori (New Zealand's indigenous people) cultural heritage. In this work, a sustainable pest management strategy to reduce *C. zealandica* damage in vineyards was proposed, highlighting the importance of understanding insect behaviour to reduce pesticide applications and thus, promote environmentally-sound agricultural systems.

Keywords: Melolonthinae behaviour, sustainable agriculture, naturally-based pest control, insect ecology.

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Chapter 1

Introduction

1.1 Sustainable agriculture and ecosystem services

The human population has been growing rapidly since 1950 and is predicted to continue on that trend in the next decades, with 9 billion people predicted by 2050 (Godfray et al., 2010). This pattern was assessed for the first time by Malthus, describing exponential population growth while food production increases linearly, predicting a human population that is always hungry (Trewavas, 2002). Thus, the gap between population growth and food quantity led scientists in the 1960s to develop a new technological agricultural approach called "the green revolution", aimed to increase worldwide food supply (Kesavan & Swaminathan, 2006; Robertson & Swinton, 2005). This technology was mainly based in the use of chemicals from fossil fuels such as fertilizers and pesticides, as well as high water use; improving crop yields (Tilman, 1999), but without reducing starvation rates (Carvalho, 2006; Reganold & Wachter, 2016). In addition, this approach revealed unwanted effects on the environment (Bouwman et al., 2013; Chindler et al., 1997; Donal, Gree, & Heath, 2001; Porter, Costanza, Sandhu, Sigsgaard, & Wratten, 2009; Reganold & Wachter, 2016; Sandhu, Wratten, & Cullen, 2010b; Tilman et al., 2001; Tilman, 1999), such as non-target species death, which was originally emphasized by Rachel Carlson's book "Silent Spring" (Carson, 1962). One of the main current concerns is biodiversity loss and its functions due to land use change (Sala et al., 2000) and climate change (De Chazal & Rounsevell, 2009). Biodiversity has a huge impact on the regulation of ecosystem functions (Fu, Wang, Su, & Forsius, 2013; Loreau, 2000), stability (McGrady-Steed et al. 1997; Tilman et al. 2006) and resilience (Chapin III et al., 2000; Folke et al., 2004), as well as on human well-being (Cardinale et al., 2012; Díaz, Fargione, Chapin III, & Tilman, 2006; Faith et al., 2010). Biodiversity therefore, is a key component in sustainable agricultural production (Altieri, 1999; Gurr, Wratten, & Snyder, 2012; Kremen & Miles, 2012; Parker, Crowder, Eigenbrode, & Snyder, 2016; Snyder, 2009). Since the 1960s, an exponential increase in scientific literature related to "sustainable agriculture" has occurred (Figure 1.1), and over that time, different types of sustainable agriculture have been proposed (De Jesus, 2005). These include natural agriculture (Fukuoka, 1978), organic agriculture (Reganold & Wachter, 2016), permaculture (Ferguson & Lovell, 2014), biological/ecological agriculture (Kremen & Miles, 2012), and agroecology (Altieri, Nicholls, Henao, & Lana, 2015; Tomich et al., 2011). One interesting approach to the sustainability concept in agriculture is the quantification, recognition and enhancement of "ecosystem services" (Costanza et al., 1997;

Kremen & Miles, 2012; Power, 2010; Sandhu, Wratten, & Cullen, 2010a; Sandhu et al., 2010b; Sandhu, Wratten, Cullen, & Case, 2008; Swinton et al., 2007; S. Wratten, Sandhu, Cullen, & Costanza, 2013). The core idea in this concept is that ecosystems contribute to human wellbeing, in terms of provision of food, water, climate regulation, cultural values and others (Costanza et al., 1997; De Groot, Wilson, & Boumans, 2002; Jax et al., 2013). Ecosystem services quantification is usually performed through adding a monetary value to ecosystem functions (Costanza et al., 2014), although sometimes this valuation might go beyond that, considering ethical, spiritual, and aesthetic valuation (Jax et al., 2013; Roberts et al., 2015; Wratten, Gillespie, Decourtye, Mader, & Desneux, 2012). In recent years, scientific evidence has emphasized agricultural practices that promote these ecosystem services (Gurr et al., 2016; Kremen, Iles, & Bacon, 2012; Kremen & Miles, 2012; Lin, 2011; Sandhu et al., 2016; Wratten et al., 2012; Wratten et al., 2013). However, monocultural agriculture is still the basis of the current global food industry (Reganold & Wachter, 2016; Tscharntke et al., 2012). Despite the large number of scientific publications (Figure 1.1) quantifying, analysing and promoting sustainable agricultural practices, these techniques are usually hard to apply at farm level, due to cultural or economic factors (Altieri & Toledo, 2011; Swift, Izac, & van Noordwijk, 2004), as well as by policy makers and economic interests that influence political decisions (Altieri & Nicholls, 2008; Reganold & Wachter, 2016). Therefore, a transdisciplinary approach is required to promote the adoption of these sustainable techniques worldwide (Aeberhard & Rist, 2009; Méndez, Bacon, & Cohen, 2013; Reganold & Wachter, 2016).

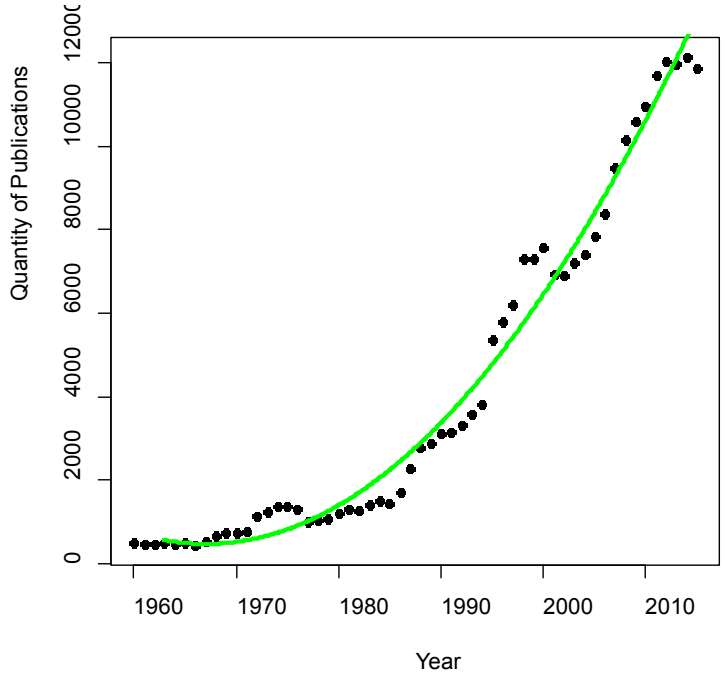

Figure 1.1. Quantity of publications related to “sustainable agriculture” found in the ISI Web of Science from 1960 to 2015, based on the same keywords used by (Gómez, Ríos-Osorio, & Eschenhagen, 2013; Kremen & Miles, 2012).

From an agronomic point of view, this change to “ecosystem service-rich agriculture” (ESRA), is leading by the gradual adoption of organic and other sustainable farming techniques, that can involve the replacement of fossil fuel inputs with organic or natural derived chemicals that produce similar effects on crop pests or in soil fertility, without significantly compromising crop yields, allowing farmers to operate without an extreme monoculture (Altieri et al., 2015; De Jesus, 2005; Gurr et al., 2016; Kremen & Miles, 2012; Reganold & Wachter, 2016). This gradual adoption of organic and other ESRA techniques allows farmers to reduce their fossil fuel based inputs, resulting in an improvement of ecosystem services produced at farm level (Gurr et al., 2016; Kremen & Miles, 2012; Sandhu et al., 2010a). Nevertheless, more scientific knowledge is needed to support the massive change from conventional agriculture to ESRA (Brussaard et al., 2010; Letourneau & Bothwell, 2008), particularly with regards to crop yield and pest management (González-Chang, Wratten, Lefort, & Boyer, 2016; Jonsson, Wratten, Landis, & Gurr, 2008; Kremen & Miles, 2012). What is needed at the farm level are service providing protocols or SPPs which simplify ecosystem service application to “recipes” which farmers can easily interpret and adopt in their respective agro-ecological units (Wratten et al., in preparation). This SPP concept has been recently defined by (Gurr, Wratten, Landis, & You, 2017) as: “A recipe that contains all the appropriate and necessary agronomic, floral and seasonal characteristics of an adequate ecosystem service provider (ESP) (Kremen, 2005) that supports a service providing unit (SPU) (Luck et al., 2009) to promote several ecosystem services” (Gurr et al., 2017). However, this SPP needs to be evaluated on each agro-ecological area to reduce the potential negative non-target effects that might generate, avoiding the production of ecosystem dis-services (Gurr et al., 2017; Zhang, Ricketts, Kremen, Carney, & Swinton, 2007). This challenge requires a trans-disciplinary approach to address cultural and technological gaps and to change the paradigm that surrounds modern fossil-fuel based agriculture (Aeberhard & Rist, 2009; Méndez et al., 2013; Robertson & Swinton, 2005; Swaminathan, 2006; Wratten et al., 2013).

1.2 *Costelytra zealandica* in vineyards

Costelytra zealandica White, 1846 (Coleoptera: Melolonthidae: Melolonthinae *sensu* Endrödi 1966) is an endemic beetle pest in New Zealand, that has been a pasture pest problem for more than 100 years (Jackson & Klein, 2006), still being the most important pasture pest in New Zealand (East, King, & Watson, 1981; Jackson & Klein, 2006; Zydenbos et al., 2011). In this agro-ecosystem, the damage is caused mainly by the second and third instar larvae feeding on pasture roots (Stewart & Stockdill, 1972). Recent pasture “improvement”, to satisfy the dairy industry, uses new cultivars of exotic species such as *Lolium* spp., *Trifolium* spp. and *Agrostis* spp., which today are the predominant

pasture species in New Zealand (Weeks, Walker, Dymond, Shepherd, & Clarkson, 2013), in addition to cultivars with new endophytes (Raman, Wheatley, & Popay, 2012). Larvae of *C. zealandica* show a marked preference for roots of these three species when compared with *Lupinus* spp., *Lotus* spp., *Phalaris tuberosa* (syn. *Phalaris aquatica*, L.), *Poa pratensis* L., *Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl, 1819, and *Holcus lanatus* L. (East & King, 1977; Farrell & Sweney, 1974a, 1974b). This preference increases the likelihood of larval survival, accentuating the damage in modern pastures.

For economic reasons, most of the pastures in the Marlborough region have been converted into vineyards (*Vitis vinifera* L.) in the past 25 years (Gabzdylova, Raffensperger, & Castka, 2009). For example, between 1990 and 2000, the area cultivated with vines increased by 100%, from 4880 to 9752 ha (Hughey, Tait, & O'Connell, 2005). *C. zealandica* is a generalist herbivorous scarab pest, capable of utilizing this new source of food, affecting both the below and aboveground plant structures of vines. The first report of adult *C. zealandica* damage in horticultural crops was in strawberry, *Fragaria* x *ananassa* Duchesne (Binfield, 1933). Adult damage was also described in kiwifruit, *Actinidia chinensis* Planch. (Blank et al., 1983), avocado, *Persea americana* Mill. (Blank et al., 1983), tamarillo, *Solanum betaceum* Cav., (1799) syn. *Cyphomandra betacea* Cav., (1899) (Blank & Olson, 1982) and blueberries, *Vaccinum corymbosum*, L. (East & Holland, 1984). In vineyards, work on this topic is scarce in relation to adult defoliation (East, Willoughby, & Koller, 1983) and larval root damage (Mundy, Alspach, & Dufay, 2005), despite the growing concern about this problem amongst winegrowers (New Zealand Winegrowers, 2013; Scarratt, 2011). In the above-ground vine plant material, defoliation can be severe, reaching in three weeks levels of 80-90% of plant defoliation (East et al., 1983). This damage is concentrated on the edges of the vineyards (Scarratt, 2011), causing complete grape loss there, with 15% of vines damaged on affected vineyard blocks (Bart Arnst, Viticulturalist, personal communication). Vine defoliation occurs between October and November in the Marlborough area (Blank & Olson, 1982; Blank et al., 1983; East et al., 1983; Farrell & Wightman, 1972; Henzell & Lauren, 1981), when adult beetles fly from their emergence sites, aggregating on plant silhouettes against the sky (Farrell & Wightman, 1972; Pottinger, 1968). This flight behaviour might be associated with the contrast that plants create against the sky at dusk, which has been suggested for *C. zealandica* (Farrell & Wightman, 1972; Kelsey, 1951, 1957, 1968; Pottinger, 1968; Radcliffe & Kain, 1971) and for other melolonthids (Durán, 1954; Schneider, 1962). Adults that land on vines (Figure 1.2) will begin to feed on leaves, shoots and inflorescences (Scarratt, 2011), eating first the uppermost leaves in the vine canopy while the lowest part of the plant is the last to be attacked (East et al., 1983), suggesting some preferences related to leaf age and associated palatability. The consumption of the leaves begins at the edge of the lamina (Blank & Olson, 1982),

leaving only the veins in cases of severe defoliation (Bart Arnst, Viticulturalist, personal communication).

Figure 1.2. Adult *C. zealandica* on a vine plant at around 21:00 h. Photo: Bart Arnst.

1.3 Rationale of the work

Until now, the most widely used management method to reduce *C. zealandica* damage in vineyards (and in several other horticultural crops (Blank et al., 1983; East & Holland, 1984)) is the prophylactic application of synthetic pyrethroids, such as lambda cyhalothrin (Karate ©) (Blank, 1992; East et al., 1983; Gabzdylova et al., 2009). These applications are made when adults are present on vine leaves, and continue for their entire flying season (Mondo Kopua, Viticulturalist, personal communication). Several problems have been seen in the past with the use of synthetic insecticides on insect pest populations (Reganold & Wachter, 2016; Tilman et al., 2001), and from an agronomical point of view, acquired resistance is the most important, (Palumbi, 2001; Roush & Mckenzie, 1987). In addition, pyrethroids are non-selective, killing the pest as well as its natural enemies (Casida, Gammon, Glickman, & Lawrence, 1983). In order to avoid the social, economic and environmental problems associated with synthetic insecticides (Reganold & Wachter, 2016; Tilman et al., 2001), it is essential to investigate sustainable pest control strategies. Thus, the severity of the damage caused by *C. zealandica* in vineyards and its current chemically-based control strategy arise as an interesting pest-crop model to investigate sustainable approaches for pest control. In addition, the New Zealand wine

industry is increasingly concerned about the environmental impacts of modern wine production, adopting the concept of "sustainability" on its management and marketing strategies (Gabzdylova et al., 2009). This sustainable approach is based on personal values that each wine company has, as well as an increasing consumer´s demand for environmentally-sound products in New Zealand (Forbes, Cullen, Cohen, Wratten, & Fountain, 2011). Nevertheless, there is no scientific information about sustainable management of adult *C. zealandica* in vineyards, or in any horticultural crop. This highlights the need for testing different ecologically-based strategies to control this endemic beetle pest.

1.4 Aims, knowledge gaps and hypotheses

1.4.1 Rearing *C. zealandica* adults under laboratory conditions from field-collected larvae

The hypothesis evaluated in Chapter 2 is:

Hypothesis 1. Adult *C. zealandica* survival rate from field-collected larvae can be improved by using novel rearing containers.

Adult *C. zealandica* is naturally available only during a short time window during the year, when mating, feeding and flight activity occur around a 4 weeks period (Farrell & Wightman, 1972; Wigley & Dhana, 1992). This reduces the amount of time to investigate aspects of its behaviour, biology and ecology. A larvae-rearing method to enhance the availability of adults, from July to late-January has been previously proposed (Wigley & Dhana, 1992). However, rearing larvae to adults of this species under laboratory conditions from field-collected larvae has historically been yielding low adult survival rates (Lefort, Boyer, et al., 2015; Wigley & Dhana, 1992). In this work, Chapter 2 presents a slight modification of the method proposed by (Wigley & Dhana, 1992). That chapter aims to achieve an increase in the number of laboratory-reared adults from field-collected larvae.

1.4.2 Adult *C. zealandica* sex-ratio throughout its flight season

The hypotheses evaluated in Chapter 3 are:

Hypothesis 2. The proportion of *C. zealandica* males is higher at the beginning of its flight season.

Hypothesis 3. *C. zealandica* females arrive before males do on vine plants.

Observations on *C. zealandica* flight dynamics have been reported before (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). However, there is no work on observing or measuring the differences

in sexes arriving at the plant foliage during its flight activity, at both, daily and monthly time scales. In Melolonthinae, sex-driven colonization patterns are different between species. For example, in *Melolontha melolonta* L., females arrive on plant foliage before males do (Reinecke, Ruther, Tolasch, Francke, & Hilker, 2002), although in *Maladera matrida* Argaman, males arrive on the plant host before females (Harari, Ben-Yakir, & Rosen, 1994). This differential sexual arrival revealed the mechanisms behind the colonization patterns within those species, which might lead to an optimised strategy to reduce their populations (Reinecke, Ruther, Mayer, & Hilker, 2006). For this reason, Chapter 3 attempts to understand the sex-driven colonization dynamics on vine plants, aiming at contributing to a better understanding of *C. zealandica* behaviour, and also to the design of a better management strategy for this pest in vineyards.

1.4.3 *C. zealandica* larvae and adult distribution in vineyards

The hypothesis evaluated in Chapter 4 is:

Hypothesis 4. Adult *C. zealandica* numbers and its larval density are higher at the edge of the blocks compared to their centre.

Within this species, larval populations in pastures present a patchy distribution in the soil (Jackson & Klein, 2006; Kelsey, 1951). This has been related to females' reproductive behaviour during their first flight phase (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). During their second flight phase females respond to a silhouette in the sky for landing, feeding and mating (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). Vineyards present different plant architecture compared to pastures therefore, vine silhouettes might influence female landing and subsequently, male colonization dynamics (Chapter 3). Adult *C. zealandica* distribution has never been measured before in any crop or natural habitat. The vine plant architecture might also contribute to the larval distribution within vineyard blocks, as larval densities in the ground have been previously associated with females' mating sites (Kelsey, 1951; Pottinger, 1968). Chapter 4 aims at presenting data on adult and larval distribution within vineyard blocks, discussing these findings in terms of adult *C. zealandica* behaviour. Understanding this pest's distribution within agricultural areas can give important insights into its management, as efforts can be concentrated were the pest is most abundant (Ferguson et al., 2003; Sivasubramaniam, Wratten, & Frampton, 1999). The aim of Chapter 4 is also to discuss results in terms of sustainable *C. zealandica* pest management.

1.4.4 Sustainable *C. zealandica* management in vineyards

The hypotheses evaluated in Chapter 5 are:

Hypothesis 5. Feeding and landing deterrents reduce adult *C. zealandica* defoliation on vines.

Hypothesis 6. Crushed mussel shells reduce adult *C. zealandica* flying and landing activity on vines, reducing its damage and increasing grape yield.

Based on the results provided in Chapters 3 and 4 about adult *C. zealandica* ecology and behaviour in vines, a sustainable approach to reduce its damage is presented in Chapter 5. This approach evaluated two feeding deterrents; hydrophobic particle films (Glenn, Puterka, Vanderzwet, Byers, & Feldhake, 1999) and diatomaceous earths (Korunic, 1998). These deterrents are silica-based dusts that can alter insect behaviour once insects land on deterrent-treated plant foliage, reducing their feeding damage on several crops. Due to their composition and underlying mechanisms behind pest control, the undesired effects of synthetically-derived pesticides on pest acquired resistance and human health are avoided (Glenn et al., 1999; Korunic, 2013; Silva & Ramalho, 2013). A combination of both deterrents has been recently evaluated to reduce the damage caused by the vine cicada *Psalmocharias alhageos* Kolenati (Homoptera: Cicadidae) in Iran (Valizadeh, Abbasipour, Farazmand, & Askarianzadeh, 2013). Also, the potential contribution of crushed mussel shells from *Perna canaliculus* Gmelin (Mollusca: Mytilidae) was investigated for its use in pest control. Recently, it has been suggested that these shells applied as mulch in the under-vine areas reflect UV-B light (Crawford, 2007; Creasy & Ross, 2010), which plays a major role in host plant recognition during insect flying, probably altering plant contrast against the sky (González-Chang, Gurr, Tylianakis, & Wratten, 2017; Prokopy & Owens, 1983). Therefore, crushed mussel shells might contribute to reducing the number of *C. zealandica* adults landing on vines, with potential effects on reducing plant damage and increasing grape yield. Mollusc shells have never been evaluated before for pest control. Thus, the aim of Chapter 5 was to evaluate these feeding and landing deterrents on adult numbers, vine damage and grape yield, as a sustainable pest control approach. Also, *C. zealandica* flying and landing behaviour was analysed when those shells were applied.

Chapter 2

An improved method to produce adults of *Costelytra zealandica* from field-collected larvae

2.1 Abstract

Rearing techniques provide a unique opportunity to study aspects of insect ecology, behaviour and physiology. Both the larval and adult stages in Melolonthinae scarabs have important impacts on crop and pasture yields worldwide. Rearing techniques for this group of phytophagous beetles usually results in a low survival rate from larva to adult, varying from 10% to 50%. Here, the current rearing method used for the New Zealand grass grub (*Costelytra zealandica*) was improved by increasing the pupation weight threshold, as well as by changing the container type used to rear the larvae. This improved method increased the survival rate from larva to adult to 83%. The technique developed here may help increase the laboratory survival rate of other Melolonthinae species worldwide.

Keywords: White grubs, laboratory-rearing methods, mass-rearing.

2.2 Introduction

The “art” of insect rearing has a history of more than 3000 years beginning in ancient China when silkworms (*Bombyx mori* L.) were reared to produce fine fabrics (Kurin, 2002). Rearing techniques also provide a unique opportunity to study the biology, life cycle and physiology of insects. Some scarab species in the subfamily Melolonthinae, commonly known as white grubs, are important pests worldwide (Jackson & Klein, 2006), reducing crop and pasture yield at the larval stage by feeding on roots (e.g., Hata et al. 2014), and in some cases by consuming plant foliage as adults (e.g., East et al. 1983; Oliveira et al. 2007). However, detailed methods of producing laboratory cultures of these animals for scientific research are rare (Romero-López, Arzuffi, & Figueroa-Brito, 2011). Amongst those few, the most frequently found rearing technique is to confine the larvae in a plastic container filled with soil and feed them with slices or cubes of plant material (e.g., Burakowski 1993; Hata et al. 2014) or directly with living roots (e.g., Miner 1948; Rodrigues et al. 2010). These containers are kept at optimal temperatures and conditions for adult production. This rearing approach has been used

for different melolonthid species, with survival rates from larva to adult varying generally from 10% to 50% (Burakowski, 1993; de Souza, Maia, Albuquerque, & Iannuzzi, 2015; Rodrigues et al., 2010; Rodrigues et al., 2008; Romero-López et al., 2011). Although, an exception for this general trend was found in one Mexican species (*Phyllophaga vetula* Horn), which had a higher survival rate (85%) when compared with other melolonthids (with survival rates from 20% to 50%), all collected in the same area (Aragón-García, Morón, López-Olguín, & Cervantes-Peredo, 2005). This suggests that the success of the method is partly species-dependent.

In New Zealand, the larvae of *Costelytra zealandica* White (Scarabaeidae: Melolonthinae), are endemic pests of pastures, and adults of *C. zealandica* have been found feeding on the foliage of horticultural crops such as blueberries, kiwifruits and vines (East et al., 1983; New Zealand Winegrowers, 2013). Despite the economic impact of such defoliation, laboratory experiments with adults are rare, mainly because of the reduced availability of adults during the flight season (i.e., from late spring to early summer), the short life-span of the adults (i.e., 3-4 weeks) and the difficulty of rearing this species under laboratory conditions (Wigley & Dhana 1992; Lefort et al. 2015a). In 1992, Wigley and Dhana suggested a technique to overcome some of these issues. They proposed the use of varying rearing temperatures throughout the developmental stages in order to accelerate or delay *C. zealandica* larval development, and consequently, adult emergence. This method extended access to adults from July to late-January. In addition, the larval weight necessary to enter the pupation phase, called hereafter, "pupation threshold" is critical for inducing morphological changes from larva to pupa, previously recorded for this species to be 120 mg (Wightman, 1974a). Despite the noticeable improvements of this *C. zealandica* rearing method in terms of adult availability and in the establishment of temperature thresholds necessary to reach each developmental stage, the highest adult emergence rate never exceeded 25% (Wigley & Dhana, 1992). In the current note, we report a modification of the method used by Wigley and Dhana (1992), which greatly improved the survival rate from larvae to adults up to 83%.

2.3 Material and methods

A total of 633 third-instar larvae were collected from Lincoln University's Field Service Centre arable land (Canterbury, NZ, 43°38'S; 172°27'E). Samples were taken from late April to mid-May 2014. Larvae were weighed and individually placed in either: (1) cylindrical plastic containers (CPCs) (2.5 x 7 cm) filled with 50 g of gamma-irradiated (Schering-Plough Animal Health, Wellington, NZ) (Lefort et al. 2015b) moist soil (20% w/w); (2) in 14-well ice cube trays (ICTs) (24.5 x 9 x 2.5 cm); or (3) in 24-well tissue culture plates (TCPs) (Biofil ®, 12.5 x 8.3 x 2.3 cm). CPCs were closed with a plastic lid with holes (Figure 2.1). A piece of organically-cultivated carrot (*c.* 1 cm^3) was put in each of the three

container types, which were then stored in a dark climate-controlled room at 15°C to increase larval weight until 180 mg, based on the results of Wightman (1974b). ICTs were wrapped with moist tissue paper and grouped in pairs inside a plastic bag, to reduce larval and carrot desiccation. In the ICT and TCP treatments, carrot was supplied every 4 to 7 days, while in CPCs it was supplied every two weeks, as larval consumption rate was lower under the latter treatment. During this feeding step, all diseased larvae were removed and the tissue paper was changed and rewetted (ICT only). After two weeks, larval weight was recorded and individuals weighing more than 180 mg were placed in a new clean CPC, ICT or TCP. All container types were placed in an incubator chamber at 4°C with 60% relative humidity in complete darkness for eight weeks to induce pupation (Wigley & Dhana, 1992). A total of 164 larvae were maintained in CPC, 175 in ICT, and 198 on TCP. After eight weeks, all the containers were stored in a dark climate-controlled room at 20°C to induce adult emergence. Adults started to emerge after two weeks in this controlled environment. A Chi-Squared test of independency was used to evaluate the effect of the container type (CPC, ICT and TCP) on adult survival. Statistical analysis was performed using R v.3.2.5 (R Core Team, 2016).

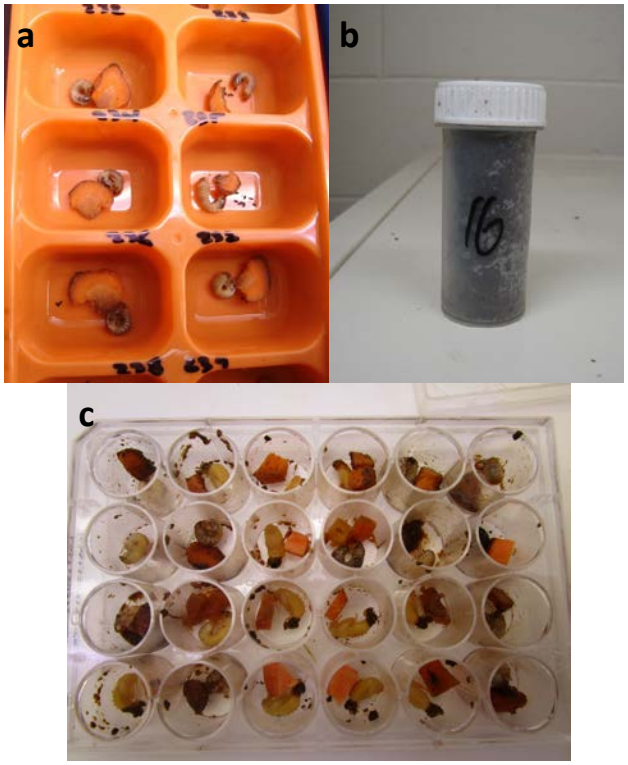

Figure 2.1. Container types used to rear *C. zealandica* larvae into adults. a) Ice cube trays, ICT; b) cylindrical plastic containers, CPC; and c) tissue culture plates, TCP. Larvae feeding on organically – cultivated carrot can be seen in a) and c). Pupae can be seen in c).

2.4 Results and discussion

The number of adults produced by each treatment was dependent to the container type (χ^2=130.3; df=2; p<0.001). A total of 38, 102 and 164 adults respectively were produced in the CPC, ICT and TCP treatments. The survival rates were 23.2, 58.3 and 82.8%, respectively. The low survival rate observed in CPC may have been due to high moisture content, either in the CPC or in the incubator chamber, as fungal hyphae were discovered in several tubes at the end of the pupation phase. Furthermore, it is important to note that because the food was placed in the top of the CPC, an additional foraging effort was needed for the larva to reach its food, which might have had potential consequences for larval weight gain and further adult development. However, a survival rate of 23.2% is within the general trends found in the literature (10% to 50%). Higher survival rates were obtained in the ICT (58.3%) and TCP (82.8%) treatments, presumably related to a higher larval weight of 180 mg before entering pupation phase, compared with the low survival rate of 25% when larvae weighing 130 mg were used to produce adults (Wigley & Dhana 1992). It has been previously observed that a direct relationship exists between larval weight and successful pupation, as the highest survival rates were obtained with larvae weighing more than 160 mg (Wightman, 1974a). This relationship is also suggested by the variations in *C. zealandica* life cycle duration; a one-year life cycle is usually present throughout New Zealand, although a two-year cycle can be found in harsh environments (Perrot & Stockdill, 1973). This variation in life cycle has been related to food and temperature restrictions, as larvae cannot gain the necessary weight to reach the pupation threshold in one season (Wightman, 1974a).

The fact that TCP produced 24% more adults than ICT was unexpected, as the methods are similar. Speculatively speaking, this result could be explained by the conditions inside the wells offered by the plate's shape (TCP), with a potential effect on gas exchange from the well with its surrounding atmosphere. When O_2 decreases due to larval respiration, the O_2/CO_2 ratio present in the plate well could change, which might trigger metabolic responses in the larva, as low O_2 levels had been linked to a temporary inhibition of ecdysteroid secretion, with a latter stimulation of the prothoracic glands that secrete ecdysone, promoting metamorphosis (DeLalio, Dion, Bootes, & Smith, 2015). It has been noted that *C. zealandica* third instar larvae produce a pupation chamber made of soil particles (Pottinger, 1968), as described for other melolonthids (Burakowski 1993; Rodrigues et al. 2010; de Souza et al. 2015), but the implications of this behaviour remains unknown. Therefore, is plausible that this pupation chamber might contribute to triggering metabolic changes at the larval stage by changing the O_2/CO_2 ratio inside it that could trigger larval metamorphosis, increasing adult emergence. Finally, considering the high survival rate found in both the ICT and TCP treatments, our study suggests that in *C. zealandica*, the use of soil (or other substrates) to produce adults from field collected larvae is not necessary. Moreover, the non-soil methods used here offer an easy approach

to larval care, with less handling effort and better control of food supply, with easy removal of diseased or dead larvae.

2.5 Conclusions

By changing the container type and increasing the larval weight before pupation phase used to rear *C. zealandica*, up to 83% of the larvae were successfully reared to adults. Our study suggests that by using ICT or TCP, a potentially valuable increase in adult emergence under laboratory conditions can be obtained from field-collected larvae. This approach, in combination with changes in temperature during the different developmental stages proposed earlier by Wigley and Dhana (1992), could facilitate laboratory studies with adult *C. zealandica* over a large part of the year, increasing our understanding of its biology and behaviour. Furthermore, the advantages provided by both ICT and TCP could eventually enhance the survival rate of other species of white grubs around the world, especially when conventional methods have produced a very restricted number of adults. However, key issues such as food type, temperature and weight thresholds must be considered from species to species, as slightly variations in these factors could affect the outcome of the culture.

Chapter 3

Ecological and pest-management implications of sex differences in *Costelytra zealandica* landing patterns on vines

3.1 Abstract

Melolonthinae beetles, comprising different white grub species, are a globally-distributed pest group. Their larvae feed voraciously on roots of several crop and forestry species, and adults can cause severe defoliation. Understanding the flight and the landing behaviour pattern within this group of insects can give valuable insights into reducing their damage. In New Zealand, the endemic scarab pest *Costelytra zealandica* produces severe defoliation on different horticultural crops. By analysing its sex ratio during its entire flight season, is clear that the proportion of males is higher at the beginning of the season, gradually declining towards its end. When adults were successively removed from the vines (*Vitis vinife*ra) at 5-min intervals after flight activity begun, the mean proportion of males ranged from 6 to 28%. The latter suggests that males were attracted to females that already landed on vines, probably through pheromone release. These results are discussed in the context of *C. zealandica* behaviour, with implications for sustainable management of this important pest group.

Keywords: Landing behaviour, vineyards, beta regression, sex-ratio.

3.2 Introduction

White grubs (Coleoptera: Melolonthidae: Melolonthinae, *sensu* Endrödi, 1966) are a widely distributed group of herbivorous insects feeding on a variety of plant hosts around the world (Jackson & Klein, 2006). Their larvae feed on plant roots, leading to severe damage to commercial crops and pastures (Frew, Barnett, Nielsen, Riegler, & Johnson, 2016; Jackson & Klein, 2006), as well as to natural forests and forestry plantations (Švestka, 2006, 2010). Although, not all species feed as adults, such as with *Leucopholis lepidophora* Blanchard (Kalleshwaraswamy, Adarsha, Naveena, & Sharanabasappa, 2016) and *Phytoloema herrmanni* Germain (Durán, 1954). In some cases minor plant defoliation has been recorded, such as with *Schizonyza ruficollis* F. (Kulkarni, Chandra, Wagh, Joshi, & Singh, 2007) and *Holotrichia* spp. (Kulkarni, Paunikar, Joshi, & Rogers, 2009) in India, *Hoplia philanthus* Füessly in Belgium (Ansari, Casteels, Tirry, & Moens, 2006), and *Phyllophaga cuyabana*

Moser in Brazil (Oliveira & García, 2003). However, dramatic defoliation can also occur. In Eastern Asia, *Ectinohoplia rufipes* Motschulsky adults can severely defoliate ornamental trees around golf courses, as well as in gardens and parks (Kim et al., 2013). In Central and Western Europe, adult defoliation by *Melolontha melolontha* L. and *M. hippocastani* F. has led to severe defoliation on different horticultural crops, such as vines (*Vitis vinifera* L.) and forest trees, respectively (Jackson & Klein, 2006; Reinecke et al., 2006; Švestka, 2006, 2010). Such defoliation has been also recorded in New Zealand, where *Costelytra zealandica* White attacks several horticultural crops, including tamarillo, avocado, blueberries, strawberries, kiwifruit, and vines (Binfield, 1933; Blank & Olson, 1982; Blank et al., 1983; East & Holland, 1984; East et al., 1983). In some cases, severe defoliation can occur, leading to the prophylactic application of synthetic insecticides to reduce damage (Blank, 1992; Blank et al., 1983; East et al., 1983). Nowadays, the use of such approach is strongly discouraged due environmental and human health problems related to their use (Reganold & Wachter, 2016).

The importance of the defoliation produced by this group of beetles has promoted investigation of their plant colonization patterns, subsequently leading to work on their flying and landing behaviour. In Melolonthinae, after landing on their host plants, females attract males by releasing sex pheromones (Henzell & Lowe, 1970) or by the release of green leaf volatiles (GLV) produced after feeding on plant foliage (Harari et al., 1994; Reinecke, et al., 2002), or both acting together (Reinecke et al., 2006; Reinecke, Ruther, & Hilker, 2002). In *Maladera matrida* Argaman, males land on their plant hosts before females do. By feeding on those, GLV are released attracting the females to where feeding occurs (Harari et al., 1994). Conversely, in *M. melolontha* and *M. hippocastani*, females arrive before males do. In that case, GLV produced by female feeding attracts males, which are also attracted by the release of female pheromones (Reinecke et al., 2006). Observations on *C. zealandica* flying behaviour have suggested that females land before males on shrubs and trees (Farrell & Wightman, 1972). After landing, males are attracted to those plants by the female pheromone, identified as phenol (Henzell & Lowe, 1970). Although the effects of this chemical on male attraction are well understood for *C. zealandica* (Henzell & Lowe, 1970; Marshall et al., 2016; Unelius et al., 2008), for *M. melolontha*, *M. hippocastani* (Reinecke et al., 2006), and *P. cuyabana* (Zarbin, Leal, Ávila, & Oliveira, 2007), there are no studies quantifying adult *C. zealandica* sex ratio at the landing phase of its daily plant colonising activity. In addition, no research has reported the trends in sex ratio throughout the entire *C. zealandica* flight season for any agricultural or natural system. We hypothesized that females arrive on *V. vinifera* before males do. Therefore, the aims of this work were: i) to quantify *C. zealandica* sex ratio throughout its seasonal and daily flight period; and ii) to correlate adult abundance with sex ratio through its seasonal and daily flight activity.

3.3 Material and Methods

3.3.1 Study sites

This work was conducted in the Marlborough region of New Zealand. In this area, two commercial vineyards were chosen. One in the Awatere Valley (41°44'S; 173°52'E) is owned by Kono Beverages, and the other close to Blenheim city (41°33'S; 173°55'E) belongs to Wither Hills. These locations are situated in homogeneous landscapes generally dominated by conventionally-managed vineyards. To reduce the effect of those management practices on the interpretation of the observations described below, organically-managed vineyard blocks (cv. Pinot Noir) were chosen at both sites. The sizes of the blocks were 6.12 and 4.58 ha in the Awatere Valley and Blenheim, respectively. During the study period, from late October to late November 2014 and 2015, no pesticides or herbicides were applied on those blocks, apart from sulphur, which was applied to control fungal diseases. In New Zealand, organic vineyard areas, including headlands, inter-row and under-vine areas are covered by a mixture of grass species. These mainly comprise ryegrass (*Lollium perenne* L.), white clover (*Trifolium repens* L.), and fescue (*Festuca* spp.).

3.3.2 Adult *C. zealandica* sampling

Sampling was carried out every day during this insect's flight season. In the Marlborough area, individuals start fly from the end of October until the end of November (Farrell & Wightman, 1972). Within this period, adults fly at dusk, 20 min after sunset, for approximately 26 min (see Chapter 5). During their flight activity, adults land on vine foliage and then feed and mate, staying on the vines for at least 3 h (González-Chang, Unpublished data). For this reason, adults were visually counted and then removed from 96 selected plants at both study locations from 21:30 until 23:00 h. These plants were distributed within three rows, from the edge of a vineyard block until 50 m towards its centre. This sampling method was performed at the Awatere Valley during 2014 and 2015, and in Blenheim only on 2015.

3.3.3 Seasonal sex ratio

Every day during the flight season, from October 26 until December 2, hand-collected beetles were put in one plastic bag. However, in 2015 flight in the Awatere Valley started on November 7. Using a 50 ml cylindrical plastic container, a sub-sample was taken from that bag for sex ratio analysis. Within this sub-sample the ratio was analysed by randomly taking 20 individuals from it. This procedure was repeated three times for each day. The proportion of males (M) over females (F) for each sampled day was obtained after taking a mean of those three measurements. This proportion was calculated as M/(M+F). Days without flight activity were removed from further sex-ratio analysis. The separation of males from females was carried out using the morphological characteristics

proposed by Kain, (1972) and Kelsey, (1965). Using a stereomicroscope (Carl Zeiss), the distal-ventral section of the abdomen was examined for male characters, including a shallow depression in the centre of the sixth abdominal sternite (Kelsey, 1965), and the presence of parameres through the pygdium (Kain, 1972). Female characters included the presence of colleterial glands, as two spherical dots in the fifth sternite, and two genital sclerites situated at each side of the vulva (Kain, 1972).

3.3.4 Adult *C. zealandica* removal to determine daily sex-ratio patterns

To investigate the dynamics of sex-ratio changes on vine foliage during the flight season, adult beetles were successively removed from the vines at different periods during 2015. At both sites, adults were removed from the vine foliage at dusk 5, 10, 15, 20 and 25 min after flight started. One plant at the edge of each of those selected vineyard blocks at each location was used for this experiment. This plant was different from the ones used in the seasonal sex-ratio determination explained above. Adults were visually counted on the selected vine and then removed by hand at each time period. When the number of adults collected exceeded 60, 20 individuals were sub-sampled three times from those samples. The mean of those proportions was used for further statistical analyses. The mean proportion of males and total adult numbers for each sampled day on each time interval were considered as replicates. This sequential adult removal was performed from November 2 to 28, and from November 14 until 28 at Blenheim and the Awatere Valley, respectively. Days without flight activity, because of adverse weather, were removed from further sex-ratio analysis.

3.3.5 Statistical analyses

The proportions obtained for each day during the sampling season, and those calculated at each evaluated time period, were analysed using a generalised linear model (GLM), with a beta-binomial distribution and “logit” as the link function (Cribari-Neto & Zeileis, 2010). It has been previously suggested that this beta regression approach is inherently heteroscedastic and easily accommodates asymmetries typically found in rates and proportions (Cribari-Neto & Zeileis, 2010; Grün, Kosmidis, & Zeileis, 2012). Beta-binomial GLMs were calculated with the R package “Betareg” (Cribari-Neto & Zeileis, 2010). Spearman’s rank correlation was used to assess the correlation between adult abundance and sex ratio through the flight season (Crawley, 2007). The same analysis was used to evaluate the correlation between adult abundance and the sex ratio at each time period studied. Differences in adult abundance between periods were analysed using Tukey’s multiple contrasts post-hoc analysis (Venables & Ripley, 2002) after fitting a GLM with a negative binomial distribution and a logarithmic link function at each sampling site. Negative binomial GLM and Tukey test were performed using the R packages “MASS” (Ripley et al., 2016) and “Multcomp” (Hothorn et al., 2016),

respectively. All statistical analyses were performed using the statistical software R v.3.2.5 (R Core Team, 2016).

3.4 Results

3.4.1 Adult *C. zealandica* abundance and sex ratio through time

Adult abundance during 2014 and 2015 is shown in Figure 3.1. In the Awatere Valley in 2014, a total of 36, 369 adults were sampled, while in 2015 only 6,111 were collected. During 2015 in Blenheim, 14,731 adults were collected. Sex-ratio was not statistically correlated with beetle abundance through the flight season in the Awatere Valley during 2014 (ϕ=0.02; p=0.92) nor in 2015 (ϕ=-0.35; p=0.12). However, at the Blenheim site, this correlation was positive and significant (ϕ=0.51; p<0.01). In the Awatere Valley the proportion of males decreased through time during 2014 (slope=-0.08; z=-4.8; p<0.001) and in 2015 (slope=-0.02; z=-2.6; p<0.01). Similar result was observed in Blenheim during 2015 (slope=-0.03; z=-4; p<0.001). Those trends are shown in Figs. 3.2-3.4.

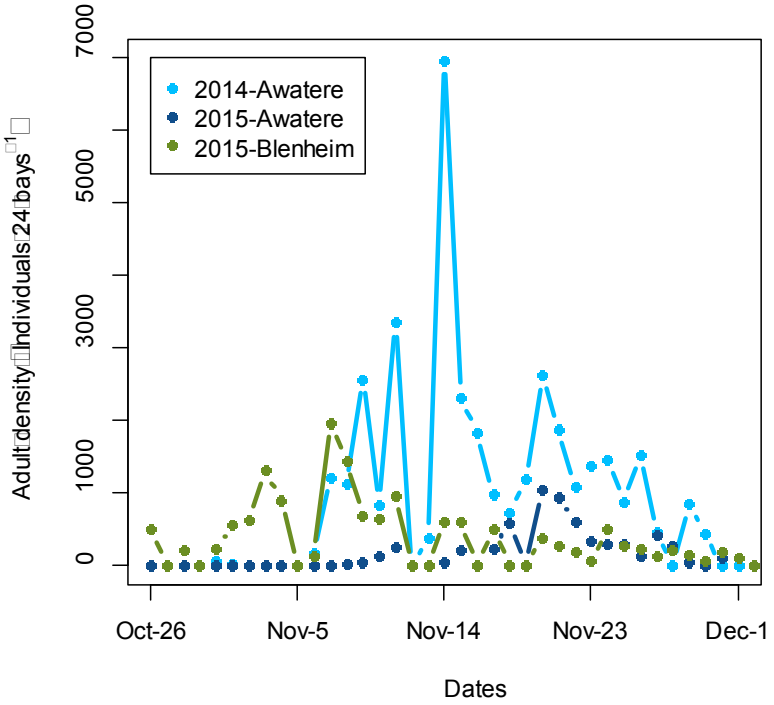

Figure 3.1. Temporal abundance of *C. zealandica* adults through the 2014-2015 flight seasons. Adults were counted and removed by hand on 24 bays (96 vines plants) every day from October 26 until December 2. In light and dark blue, adult distribution through time is presented for Awatere Valley in 2014 and 2015, respectively. In green, adult distribution for Blenheim in 2015 is shown.

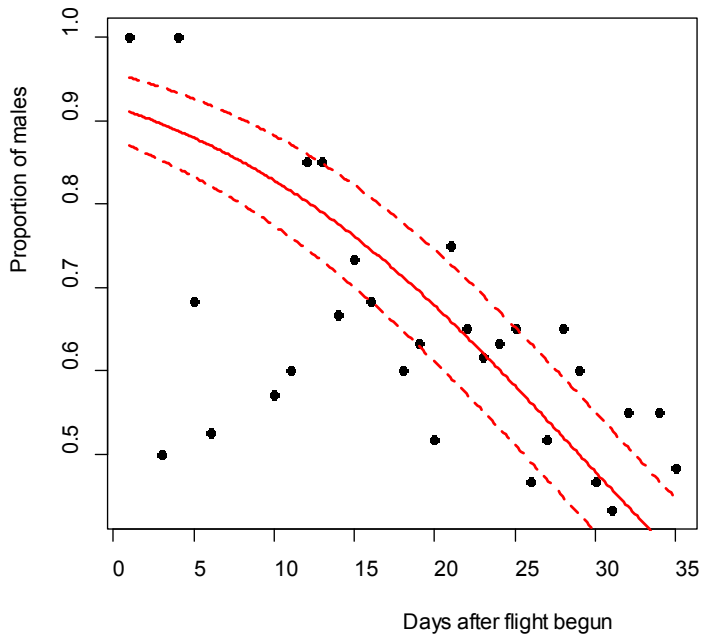

Figure 3.2. *C. zealandica* sex ratio (proportion of males) on 35 days after its flight begun, from October 27 to November 30 at the Awatere Valley in 2014. The beta regression model (solid line) is presented with its confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-0.08; z=-4.8; p<0.001).

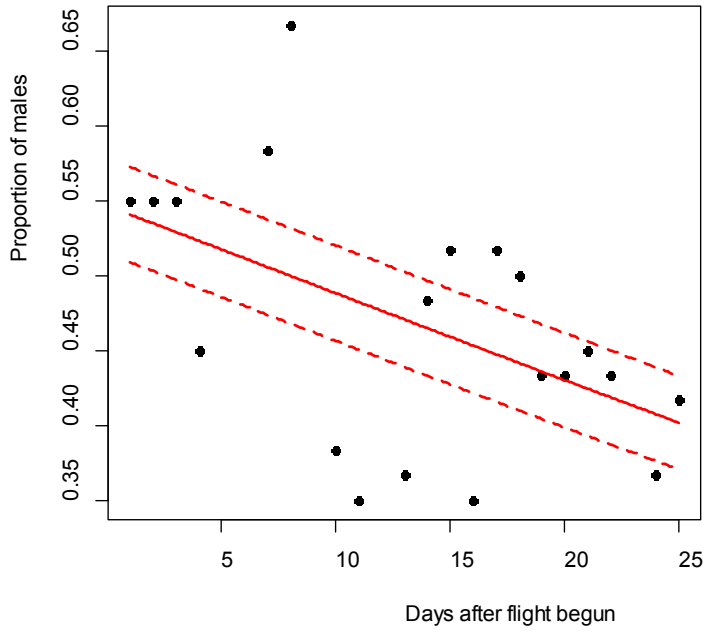

Figure 3.3. *C. zealandica* sex ratio (proportion of males) on 25 days after its flight begun, from November 7 to December 1, at the Awatere Valley in 2015. The beta regression model (solid line) is presented with its confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-0.02; z=-2.6; p<0.01).

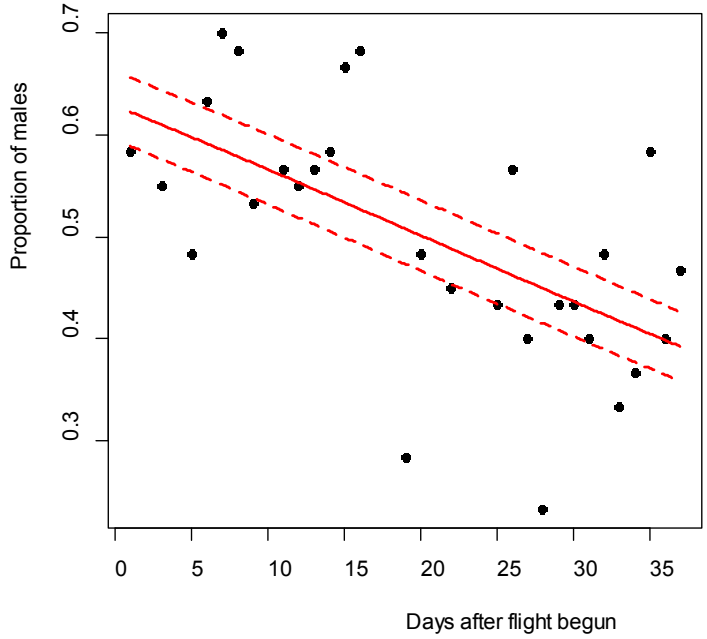

Figure 3.4. *C. zealandica* sex ratio (proportion of males) on 37 days after its flight begun, from October 26 to December 1 close to Blenheim city (2015). The beta regression model (solid line) is presented with its confidence intervals (dashed lines). The proportion of males is reduced through time (slope=-0.03; z=-4; p<0.001).

3.4.2 Adult removal and daily sex-ratio trends

When adults were removed from the vine foliage every five minutes after their flight activity started, the proportion of males increased through those (slope=0.06; z=2.12; p<0.05) at Blenheim but not at the Awatere Valley (slope=0.001; z=0.03; p=0.98). These trends are shown in Figure 3.5. Adult abundance at each period for the two studied locations is shown in Figure 3.6. Tukey contrasts showed that adult numbers significantly increased between 10 and 15 min after flight occurred, and then decreased to initial levels between 20 and 25 min after it started. In the Awatere Valley, flight activity completely ceased 25 min after began, while in Blenheim it stopped 30 min after it began. No correlation was found between adult abundance and the sex-ratio during each time period evaluated at Blenheim (ϕ=-0.1; p=0.87) and in the Awatere Valley (ϕ=-0.8; p=0.2).

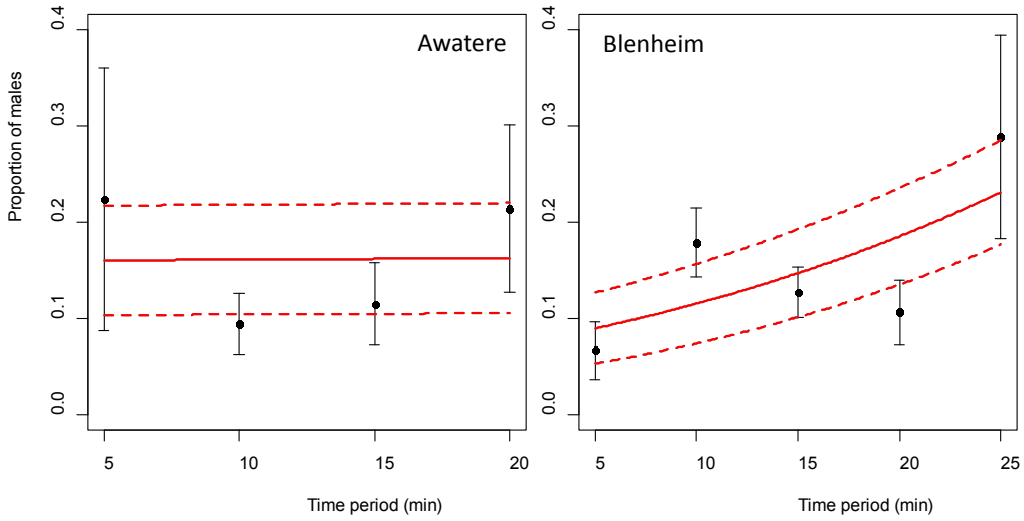

Figure 3.5. Proportion of *C. zealadica* males, after adult removal from a single vine plant at five-minute periods after daily flight activity begun, at the Awatere Valley (right) and Blenheim (left). The beta regression model (solid line) is presented with its confidence intervals (dashed lines). In the Awatere Valley, male proportion did not change during *C. zealandica* daily flight activity (slope=0.001; z=0.03; p=0.98), but in Blenheim, it increased through this time period (slope=0.06; z=2.12; p<0.05). Error bars are two-standard errors.

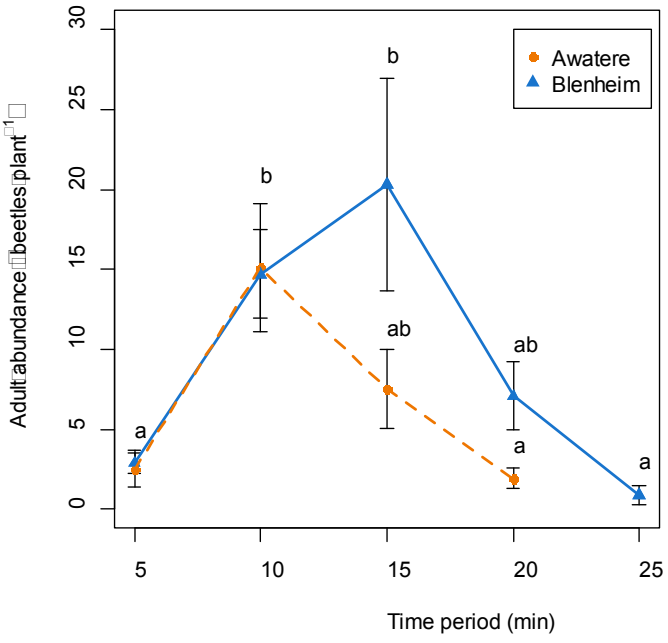

Figure 3.6. Adult abundance during *C. zealandica* daily flight activity at different five-minutes periods, at the Awatere Valley (circles) and Blenheim (triangles). Tukey contrasts are presented as different letters above each dot. Different letters represent significantly differences between the studied periods, but not between locations. Error bars are two-standard errors.

3.5 Discussion

In this work, seasonal and daily changes in adult *C. zealandica* sex-ratio throughout its flight season are presented for the first time. The proportion of males significantly decreased towards the end of the season at both studied locations. However, when adults were removed from the vine foliage at several time periods during the same day, trends in the sex-ratio differed between the two locations. These results are discussed in terms of this insect's behaviour. The implications of these results on sustainable *C. zealandica* control in vineyards are also discussed.

3.5.1 Seasonal sex-ratio trend

A widely-accepted sex-ratio theory based on natural selection proposed by R.A. Fisher, suggests that sex-ratio is equilibrated on a 1:1 proportion to promote species survival (Fisher, 1930; p.143). However, many species do not follow this trend as they respond to their changing local environmental conditions (Hamilton, 1967). In Melolonthinae (*sensu* Endrödi, 1966) sex-ratio though their flight season can be female-biased (Kalleshwaraswamy et al., 2016; Méndez-Aguilar, Castro-Ramírez, Alvarado, Pacheco-Flores, & Ramírez-Salinas, 2005), male-biased (Ansari et al., 2006; Švestka, 2006, 2010) or at a 1:1 ratio (Harari et al., 1994; Méndez-Aguilar et al., 2005). However, it is worthwhile noting that sex-ratio can vary between those proportions every day during the flight season (Kalleshwaraswamy et al., 2016; Švestka, 2006, 2010), as shown in Figures 3.2-3.4. In this work, seasonal sex-ratio varied between years and locations, being male-biased (64% males) and female-biased (47% males), in the Awatere Valley in 2014 and 2015, respectively. In Blenheim, the seasonal sex-ratio was 1:1 (50% males). This highlights the temporal and spatial variability of melolonthid sex-ratio, which might be determined by environmental conditions (Gempe & Beye, 2011; Sánchez, 2008). Previous studies suggested that *C. zealandica* males appear before females do (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). This might have important evolutionary consequences for the survival of this species because as soon as the females emerge, males are already present for mating (Durán, 1954; Harari et al., 1994; Kalleshwaraswamy et al., 2016; Kelsey, 1951). This is in agreement with the results presented in this work, as higher proportion of males was found at the beginning of the flight season at both studied years and locations. Because those adults were removed from the plants, the reduction in male numbers through time might be associated with a reduction in female pheromone production towards the end of the flight season, as its effects on male attractiveness have been well studied in *C. zealandica* (Henzell, 1970; Marshall et al., 2016; Unelius et al., 2008). Recently, it has been suggested that phenol, the main sex pheromone in *C. zealandica* (Henzell, 1970), is produced by a mutualistic bacterium *Morganella morganii* in its colleterial gland, which is biosynthesized from the amino acid tyrosine (Marshall et al., 2016). In general, insect protein synthesis decreases with aging (Levenbook, 1986), and tyrosine in particular has been shown to decrease with age in *Bombyx mori* (Lepidoptera: Bombycidae) (Osanai & Kikuta,

1981). Considering that the *C. zealandica* flight season lasted for around 4 weeks, from late October to late November at the studied vineyards, females that landed on the vine foliage at the end of that period might already have ceased to synthesize their pheromone due to aging, reducing the number of males attracted to them.

In the Awatere Valley, no correlation was found between adult numbers throughout the flight season and changes in the sex-ratio. However, in Blenheim, this correlation was positive and significant. This suggests that an increase in adult numbers is due an increase in the number of males, because in the Awatere Valley 2014 flight season, 63% of the sampled adults at their peak in abundance (November 14, 6968 individuals) were males. Conversely, at the same location during 2015, only 37% of the adults were males at their peak (November 19, 1039 individuals). In Blenheim, 55% of them were males at their peak (November 6, 1966 individuals). The lack of any significant correlation between sex-ratio and adult abundance through time in the Awatere Valley, in contrast with the results found in Blenheim, suggests that temperature might be involved in this species sex-ratio determination at the landscape level. It is well accepted that sex determination in insects is governed by the *Doublesex* gene expression, which is affected by zygotic, maternal or environmental signals (Gempe & Beye, 2011; Sánchez, 2008). Amongst these environmental signals, temperature has been related to changes in population sex-ratio in *Sciara ocellaris* (Diptera: Sciaridae) (Nigro, Campos, & Perondini, 2007) and in the crustacean *Daphnia magna* (Cladocera: Daphniidae) (Kato, Kobayashi, Watanabe, & Iguchi, 2011). Perhaps, different temperature regimes affected the sex-ratio present at landscape level, producing a male-biased population in the warmer area of Blenheim. However, so far there is no literature related to the effects of temperature on sex determination affecting melolonthid eggs, larvae or pupae therefore, the factors underlying the contradictory results found in this work between the Awatere Valley and Blenheim in terms of adult *C. zealandica* abundance and sex-ratio correlation throughout its flight season, still remain unknown.

3.5.2 Adult removal and daily sex-ratio trends

C. zealandica flight activity has been divided in two phases (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). Firstly, males emerge from the ground and hover over the grass. When females emerge, they climb to the top of a grass leaf and release their sex pheromone to attract males. Once mating has occurred, females return to the ground to lay eggs. Secondly, females fly from the grass, searching for silhouettes in the sky, landing on trees, hedgerows and shrubs (Farrell & Wightman, 1972). After landing occurs, pheromones are released, attracting males from the surrounding areas again. Then, males and females drop to the ground, where females lay eggs, although in fewer numbers compared to their first ovisposition made close to their initial emergence sites (Farrell & Wightman, 1972). The results presented here are in agreement with the colonization sex-driven

dynamics for the second flight phase observed during 1970s, in which females land first on the vegetation; as the proportion of males found when adults were removed from the vines never exceed 28%. In fact, 10-min after flight begun, only 9 and 18% of the removed adults were males, at the Awatere Valley and Blenheim, respectively. It seems that without females, the number of males landing on vines is further reduced. Remarkably, an identical two-phase flight behaviour has been described before for *Phyllopertha horticola* L. (Coleoptera: Rutelinae) (Schneider, 1962) and for *P. herrmanni* (Durán, 1954). Similar landing behaviour where female melolonthids arrive before males on plant foliage has been also reported on *M. melolontha*, *M. hippocastani* (Reinecke et al., 2002) and *P. cuyabana* (Oliveira & García, 2003). In the Awatere Valley (2015), the proportion of males did not change throughout their daily flight activity, which lasted for 25 min. However, in Blenheim (2015) this proportion increased throughout their daily flight period, which totally ceased 30 min after flight begun. These differences in adult abundance and flight length at dusk could be related to the two-fold increase in adult numbers found in Blenheim compared to the Awatere Valley. Basically, higher female densities attracted higher amount of males. This appears to be true at Blenheim, as higher adult abundances were related to higher number of males. In addition, potential variations in the sex-ratio present at landscape level might contribute to the increase of males towards the end of the daily flight activity at Blenheim.

3.5.3 Female behaviour and plant silhouettes

Considering that males are attracted to females that have already landed on their host plant, through pheromone communication (Henzell & Lowe, 1970) and/or plant volatiles (Reinecke et al., 2002), the mechanisms behind the location of females' host plants have relevance to pest management. Different authors have proposed that female host finding in Melolonthinae is through the visual recognition of the tallest plant silhouettes in the sky (Durán, 1954; Farrell & Wightman, 1972; Oliveira & García, 2003; Schneider, 1962). Although it cannot be neglected that males might also use visual cues in the sky for orientation at some extent, a study in Brazil showed that *P. cuyabana* females preferred tall soybean and corn plants for landing and mating (García, Oliveira, & de Oliveira, 2003). Recent work suggests that plant location by *C. zealandica* females might be mediated by the contrast that plant silhouettes produce in the sky (see Chapter 5). Thus, investigating the addition of tall plant species at the edge of crops might divert females from landing further into the crop area, and also pulling the males away from it as a consequence.

3.5.4 Implications for sustainable *C. zealandica* management in vineyards

Recently, it has been suggested that a "push-pull" strategy (Khan, Midega, Pittchar, Pickett, & Bruce, 2011) might contribute to the control of white grub populations (Dynastinae, Rutelinae, Melolonthinae) around the world (Frew et al., 2016). In the context of *C. zealandica* pest control in

vines, it seems straightforward to use the sex attractant pheromone (phenol) to "pull" the adults away from the vines. This pheromone, combined with another management intervention which "pushes" the adults away, as recently shown when crushed mussel shells were applied on the under-vine vineyard areas (see Chapter 5), might arise as a novel variant on the "push-pull" approach to control this New Zealand pest in several horticultural crops. However, the use of phenol in such a way has some shortcomings. Although it was identified as the pheromone for this species more than 40 years ago (Henzell & Lowe, 1970), it has been occasionally used to control this pest due constraints in trap design that led to low capture efficiency (Unelius et al., 2008). Another negative factor in this approach is its high toxicity for humans (Shadnia & Wright, 2008; Unelius et al., 2008). Instead, if females can be attracted to a host plant outside the crop, the overall numbers of adult *C. zealandica* landing on the crop could be reduced, with subsequent consequences for reducing plant damage (see Chapter 5). The mechanisms behind female *C. zealandica* host location remain unknown, with literature only suggesting that females might locate its host plant by their contrast against the sky at dusk (Farrell & Wightman, 1972). Despite those assumptions, it cannot be ignored that females can also be attracted to different floral plant volatiles, as demonstrated for *P. horticola* (Ruther, 2004). Furthermore, the potential contribution of green leaf volatiles (GLV), released after females feed on vines (Reinecke et al., 2002), might play an important role on seasonal *C. zealandica* peak activity, as it has previously been suggested that its damage in vines is dealt at the middle of the flight season (East et al., 1983). In this work, the peak of abundance was registered during that period. Thus, the chemical ecology underlying *C. zealandica* reproductive behaviour needs further investigation. Another approach could evaluate the contribution of native New Zealand vegetation outside the vines (Shields, Tompkins, Saville, Meurk, & Wratten, 2016), as it can provide the necessary height for females to "*look at*", and eventually release some organic volatiles that might further attract males or females. Although the establishment and growth of those native plants in horticultural areas will take time, it could contribute for a long-term sustainable *C. zealandica* pest management strategy, with effects on local biodiversity and biological conservation that need to be further addressed.

3.6 Conclusions

The proportion of *C. zealandica* males landing on the vine foliage decreased throughout the flight season during 2014 and 2015, at two New Zealand locations. Only at Blenheim (2015), increases in sex-ratio (male-biased) were correlated with increases in overall adult abundance. This might be related to spatial and temporal landscape variations in sex-ratio between Blenheim and the Awatere Valley, although those were not measured here. When adults were removed every day from the

vines at several times after their flight activity begun, female-biased sex-ratio was recorded. This suggests that females land on the plant foliage before males do, subsequently attracting them afterwards by the release of their sex pheromone, phenol. The vine colonization sex-driven dynamics during adult *C. zealandica* flight activity presented in this work might contribute to reducing the damage of this endemic New Zealand pest on several horticultural crops, and eventually in other scarabaeids with similar flight behaviour around the world.

Chapter 4

Distribution of *Costelytra zealandica* in vineyards: Implications for reduced pesticide inputs

4.1 Abstract

Information on larval and adult distribution within a crop can provide valuable insights into insect behaviour, with potential applications to sustainable pest control. In New Zealand, vines can suffer from severe damage produced by adults of the endemic scarab *Costelytra zealandica* White (Melolonthinae). Here, larval and adult distribution of this pest has been assessed by recording larval numbers in the soil and by counting and removing adults that landed on the vine plants. Also, soil moisture and soil penetration resistance were measured to evaluate their impact on larval distribution within vineyard blocks. Distribution patterns within vineyard blocks were evaluated using generalised linear mixed-effects models. This study shows that both larval and adult stages were concentrated at the edge of the vineyard, with higher larval numbers in the under-vine compared to the inter-row areas. Soil parameters partly explained differences in larval densities within the blocks. Larval spatial distribution was mainly attributed to an ancient relict adult behaviour that led to the adults' landing on tall plants to feed and mate, then laying their eggs in the under-vine after dropping from the vine plants. Based on these results, protocols can be developed for non-pesticidal or restricted pesticide management of this pest, as well as for other species displaying similar crop colonization patterns or behaviours.

Keywords: Crop aggregation, relict adult behaviour, sustainable management.

4.2 Introduction

The spatial distribution of white grubs (Coleoptera: Melolonthinae) at broader regional scales has been studied for several species in different parts of the world (e.g., Barratt 2007; Montreuil 2008; García-López et al. 2012). However, the spatial distribution at local or farm scale has only ocasionally been described. Work has focussed essentially on white grub larval distribution, bacause the damage to plant roots caused by larvae can be severe, having impacts on crop yield and plant survival (Allsopp & Bull, 1989; Ghaioule, Lumaret, Rochat, Maatouf, & Niogret, 2007; Kim et al., 2013; Pardo-

Locarno & Montoya, 2007; Way, Mwelase, Magagula, & Matimba, 2013). The understanding of larval distribution within agricultural areas can enhance the effectiveness of control strategies, as agricultural management can thereafter be concentrated on highly infested areas. For example, in Australia, the larval population of *Antitrogus parvulus* Britton (Coleoptera: Scarabaeidae) was concentrated under the row of sugarcane (*Saccharum* sp.), which was related to soil conditions, such as soil compaction and moisture content, that could affect larval survival in this agricultural system (Logan, Allsopp, & Zalucki, 2003). Several studies have shown that changes in soil condition, such as soil moisture and compaction are likely to affect larval survival (Cornelisse & Hafernik, 2009; East et al., 1981; Ellsbury, Exner, & Cruse, 1999; Frew et al., 2016; Stewart & Van Toor, 1986). Some agricultural practices contribute to increased soil compaction, affecting water and air fluxes in the soil profile (Horn, Domżał, Słowińska-Jurkiewicz, & van Ouwerkerk, 1995). Amongst those, vehicles' wheels can severely alter soil conditions (Hakansson, Voorhees, & Riley, 1988; Horn et al., 1995). In the USA, this variable has been suggested as the main factor influencing corn rootworm larvae (*Diabrotica virgifera* Le Conte) (Coleoptera: Chrysomelidae) survival and migration from soil previously planted with corn (*Zea mays* L.) to corn strips (Ellsbury et al., 1999). Despite the unquestionable importance of soil conditions on larval survival (Frew et al., 2016), a localised larval distribution within agricultural areas can also be related to adult reproductive behaviour. In Brazilian corn and soybean (*Glycine max* L.) fields, the larval population of *Phyllophaga cuyabana* Moser (Coleoptera: Scarabaeidae) was concentrated at the edge of those crops (García et al., 2003). This was explained by a direct correlation between the location of mating and oviposition sites, showing that larval spatial distribution is related to females' landing preferences for feeding and mating (García et al., 2003). This aggregated distribution in the soil has been recorded for other melolonthids colonizing a wide range of different crops, such as sugarcane in Swaziland (Way et al., 2013) and Australia (Allsopp & Bull, 1989), corn in Brazil (Oliveira & García, 2003), and even turf in Korean golf courses (Kim et al., 2013).

In New Zealand, the endemic scarabeid *Costelytra zealandica* White (Coleoptera: Melolonthinae) has been an important pasture pest for more than 100 years (Jackson, 1990; Kelsey, 1951). In this agro-ecosystem, larvae tend to be aggregated (Jackson & Klein, 2006; Kelsey, 1951), which can be explained by eggs laid in a clustered pattern by females, close to their mating site (Farrell & Wightman, 1972). During the 1980s, an increase in the number of scientific articles reporting *C. zealandica* adults attacking different horticultural crops, such as avocado (*Persea americana* Mill.), tamarillo (*Cyphomandra betacea* Cav.), kiwifruit (*Actinidia chinensis* Planch.) (Blank et al., 1983), blueberries (*Vaccinum corymbosum* L.) (East & Holland, 1984) and grape vines (*Vitis vinifera* L.) (East et al., 1983) were published. Amongst these horticultural crops, vine plants can be severely damaged by adults, reaching in some cases 100% defoliation (personal observation). The widely used approach

to reduce adult damage in conventionally managed vineyards is by prophylactic applications of synthetically derived pyrethroids (Blank, 1992), which besides the associated health and environmental problems, can deteriorate the "clean and green" image of New Zealand wines around the world (Gabzdylova et al., 2009). Conversely, larval damage is seen as a problem only on recently established young vine plants (Mundy et al., 2005), so no control methods for larvae are used on already established vineyards. A similar effect of melolonthid larvae damaging recently established oak trees (*Quercus suber* L.) in Morocco has resulted in unsuccessful tree regeneration programmes on degraded deforested areas (Ghaioule et al., 2007).

This recent adult damage caused to New Zealand vineyards could be related to recent land use changes (Jackson et al., 2012) from pastures to vineyards in the last 25 years, especially in the Marlborough area. Furthermore, the different plant structure provided by vine plants (as compared to grass) could be more "attractive" for *C. zealandica* as it has been suggested that adults fly towards the highest plant silhouette available, as an expression of relict adult behaviour (Farrell & Wightman, 1972) related to this endemic insect's original grass/shrub habitat (Merton, 1980). Nevertheless, the factors responsible for this recent vineyard invasion by *C. zealandica* adults, and for other melolonthids in different human-associated landscapes (Kim et al., 2013; Wagenhoff, Blum, & Delb, 2014) is beyond the scope of this paper, (but see Lefort et al. 2015 for suggestions). Unfortunately, there is no study so far that evaluates larval and adult melolonthid distribution within vineyards in New Zealand or anywhere else around the world. Therefore, the aims of this paper are to quantify and interpret adult and larval distribution patterns to help in managing this pest. The steps followed in this study were: i) to analyse the larval and adult distribution of *C. zealandica*, ii) to record adult seasonal flight abundance during 2014 and 2015, iii) to measure soil moisture and penetration resistance, and iv) to evaluate adult patterns along the vine row in 2014 and 2015.

4.3 Material and methods

4.3.1 Study sites

All data were collected in the Marlborough region, New Zealand. In 2014, larval and adult distributions were evaluated in the Awatere Valley (Kono Beverages, 41°44'S; 173°52'E). During 2015, adult distribution was measured in the Awatere Valley and in Blenheim (Wither Hills, 41°33'S; 173°55'E). Both sites were 50 km apart. The Awatere Valley is influenced by the Blue Mountain range, making the area colder than Blenheim. Organically-managed blocks of the Pinot Noir variety were chosen at the Awatere Valley and Blenheim for the experiments described below. As part of the viticultural practices, the inter-row and headlands were covered by grass, generally a mixture

between clover (*Trifolium repens* L.), ryegrass (*Lolium perenne* L.), and fescue (*Festuca spp*.). No pesticide or herbicide was applied on the studied plots. Marlborough is an important area for wine production in New Zealand, and both locations occur in a vineyard-dominated landscape, with few remnants of native vegetation around the vineyard properties. A range of different management techniques, including conventional, organic, biodynamic and mixed situations between those can be found in this agricultural mosaic.

4.3.2 Larval sampling

In July 2014, larval densities were measured at the edge and centre of a vineyard block, in the Awatere Valley. The "edge" was considered to be the first 15 m from the border of this vineyard block. Samples were taken at the edge and after 100 m towards the centre of the vineyard, to assess larval abundance. To do this, a spade (19 cm wide) was used to dig holes (19 x 19 cm, 25 cm depth) in the under-vine and in the inter-row, at both edge and centre. This data were expressed as larvae m^{-2}. In this experiment, three replicates were used with a total of 240 samples, with each replicate of 80 samples (n=20 holes x 3 replicates x 2 places (edge/centre) x 2 areas (under-vine/inter-row), Figure 4.1). Replicates had a regular distribution with 10 m separating them.

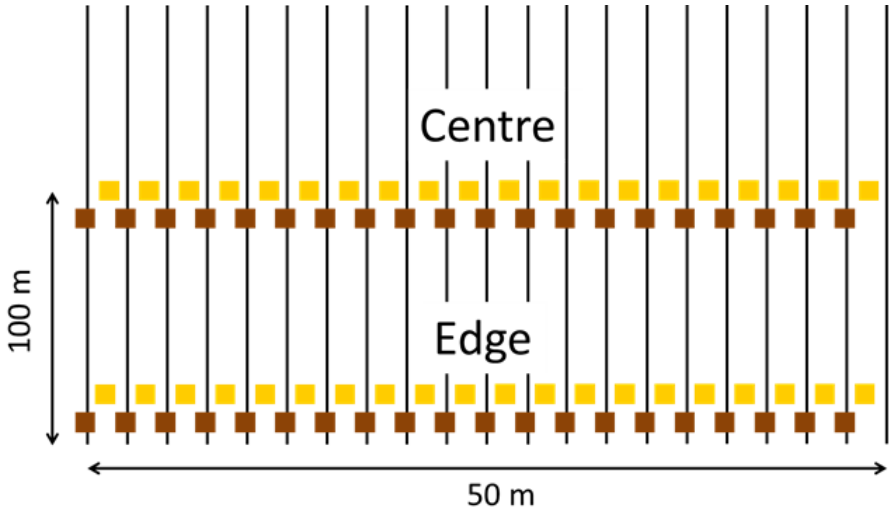

Figure 4.1. Schematic representation of one replicate (from a total of three) to sample larval densities and soil condition at the edge and centre of a vineyard block in the Awatere Valley. 20 samples were taken in the under vine and 20 in the inter-row. The edge and centre were separated by 100 m. Each replicate considered was separated by 10 m.

4.3.3 Soil condition

Soil condition was assessed by measuring the volumetric moisture content (MC) (volume %) and penetration resistance (PR) (kPa) in the under-vine and inter-row areas, at both edge and centre of a vineyard block in the Awatere Valley in July, 2014 (Figure 4.1). MC was measured using a digital soil moisture unit (Delmhorst Instruments Co, Towaco, USA). The probe was inserted at a depth of 5 cm. Soil PR was used as measure of soil compaction, as an increase in PR is due to an increase in bulk density and a reduction in macroporosity, both being direct consequences of soil compaction (Hakansson et al., 1988; Horn et al., 1995). These PR values were obtained by a hand penetrometer (Eijkelkamp, Agrisearch Equipment, Giesbeek, The Netherlands) with a 3 cm^2 cone. This cone was inserted 15 cm into the soil. All measurements were performed adjacent to each hole dug to quantify larval density within the vineyard block. MC and PR data were obtained in the under-vine and inter-row, at both edge and centre of the studied vineyard block. A total of 240 measurements for each evaluated soil parameter were obtained.

4.3.4 Adult sampling

The pattern of adult distribution on vine plants through the flight season was obtained by counting and removing by hand all adult beetles landing on 96 vine plants (3 rows of 32 vine plants each), collected every day. Collection was performed at both locations (Awatere Valley and Blenheim) during 2014 and 2015, from October 26 until December 2. This period was chosen based on the seasonal flight length for *C. zealandica* recorded previously in this area (Farrell & Wightman, 1972), but also on observations made by the authors in the field. At dusk, *C. zealandica* starts to fly (Farrell & Wightman, 1972; Kelsey, 1951), like other melolonthids around the world (García et al., 2003; McQuate, 2013; Santos & Ávila, 2009; Tanaka et al., 2008). After landing on the plants, the adults remain on the leaves, where they feed and mate at least until 23:00 h (González-Chang, Unpublished data, Figure 1.2). Then, they drop to the ground, where the females dig to lay their eggs (Blank, 1992; Farrell & Wightman, 1972). This provided a "window" between 20:30 and 23:00 h during which individuals were visually counted (García et al., 2003; McQuate, 2013) and removed by hand from the plants.

To analyse adult beetles' distribution pattern along the vine row, three vine rows were selected and adult beetles on the vine plants were counted and removed. The collection started from the first plant at the edge of the block up to 52.5 m towards its centre. Three dates in each year and location were used to analyse the distribution of adults along vine rows, in the beginning, at the middle and the end of the flight season. In 2014, the dates for the Awatere Valley were: November 7, 11 and 22, and in 2015 were November 10, 19 and 30. In 2015, the Blenheim dates were: October 26, November 2 and 16. In total, 864 vine plants were assessed over both years and sites ($n_{total}=n_{2014}$(32

plants x 3 vine rows x 3 dates x 1 site)+n_{2015}(32 plants x 3 vine rows x 3 dates x 2 sites)). Adult density between the edge and centre of the vineyard block, on two different dates: November 8 and 22 (2014), was assessed by counting and removing the adults at the edge and at 100 m towards the centre of the vineyard block. Five different rows, with a total of 10 plants on each row were sampled, at both edge and centre. Each replicate included 20 plants (10 in the edge and 10 in the centre). Replicates were separated by 10 m between each other. For this experiment, five replicates were considered, with a total of 100 plants evaluated (n=10 plants x 5 rows x 2 places (edge/centre)).

4.3.5 Statistical analyses

All analyses were performed using generalised linear mixed-effects models (GLMM), to quantify group variability under a pseudoreplicated design (Bolker, 2015; Bolker et al., 2009). A negative binomial distribution with a logarithmic link function was used when larval densities were evaluated, due to the number of zero counts and the overdispersion present (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Larval abundance in the under-vine and the inter-row (rows) within the edge and centre of the vineyard (place) were considered as fixed effects. The areas where holes were dug within a replicate were considered as a random effect. Soil conditions were evaluated using a Gamma distribution with an inverse link function (Bolker, 2015). Fixed and random effects were the same as those used when larval abundance within vineyard blocks was evaluated. Adult distribution between edge and centre over the two dates was also analysed using a negative binomial distribution with a logarithmic link function to correct for overdispersion. In this model, edge and centre (place), and the two different dates (November 8 and 22) were considered as fixed effects. Each plant within a replicate from which adults were counted and removed was considered as a random effect. When adult density was analysed along the vine rows, a penalized quasi-likelihood approximation (PQL) was used, with Poisson distribution and a logarithmic link function (Bolker et al., 2009; Zuur et al., 2009). In the latter, the position of the sampled plants in the row from the edge to the centre (plants) and the three different dates were considered as fixed effects, while the row in which those plants occurred was treated as a nested random effect (plants within a row) (Zuur et al., 2009). One PQL-GLMM was used for each location and year. For graphical purposes, a regression with its respective 95% confidence intervals was fitted for each date, location and year, based on the predicted values produced by the individual PQL-GLMMs (which already included the effect of the fixed and random effects) using a generalised linear model (GLM) with Gamma distribution and an inverse link function. All models described above were graphically checked for unusual patterns in residuals vs. fitted values, residuals within a Q-Q plot and residuals' frequency distribution (Bolker, 2015; Zuur et al., 2009). Also at this stage, overdispersion was tested again by comparing the sum of the squared Pearson residuals to the residual degrees of freedom (Bolker, 2015). A Wald test based on a χ^2 distribution was used to obtain P-values to assess the significance of the three different dates

and the interaction between those dates and adult distribution along vine rows (Bolker et al., 2009). All statistical analyses were performed using R v. 3.2.5 (R Core Team, 2016), using the R packages MASS (Ripley et al., 2016), Matrix (Bates & Maechler, 2016), lme4 (Bates et al., 2016) and aod (Lesnoff & Lancelot, 2015).

4.4 Results

4.4.1 Larval distribution

Larval abundance was higher at the edge of the vineyard block when compared to its centre (z=5.37; p<0.001). Also, higher larval numbers were found under vines compared to the inter-row (z=3.35; p<0.001). The mean number of larvae m^{-2} was 63.7 and 34.6 for the inter-row in the edge and centre of the vine block, respectively. For the under-vine, the mean larval number was 103.8 and 49.3 larvae m^{-2} for the edge and the centre, respectively. These results are shown in Figure 4.2.

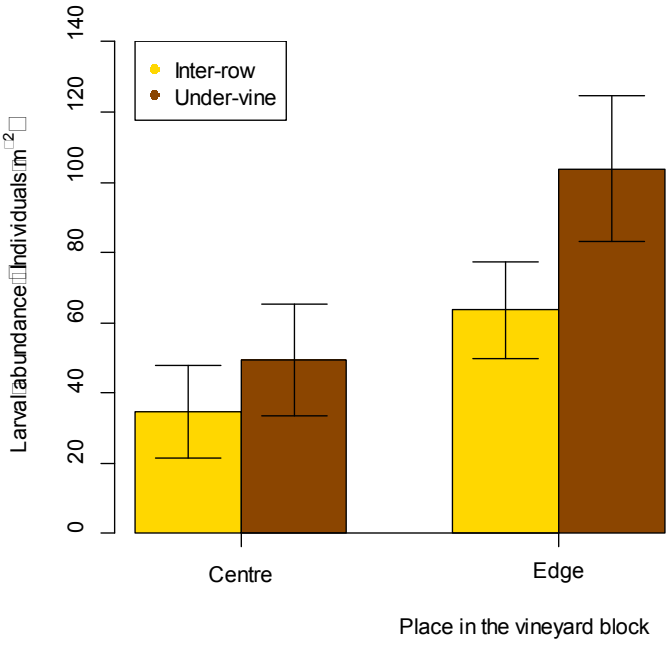

Figure 4.2. Larval abundance in the under- and inter-vine row at the centre and edge of a vineyard sampled during winter 2014 at the Awatere Valley. Confidence intervals (95%) are presented as error bars (two-standard errors).

4.4.2 Soil condition

Statistically higher soil MC values were obtained from the inter-row when compared to the under-vine (z=-16.6; p<0.001), with higher MC content at the edge of the block when compared to its centre (z=3.96; p<0.001). No interaction between these factors was observed (z=-1.08; p=0.28). The mean MC values for the inter-row were 33.9% and 31.5% for the edge and centre, respectively. Under the vines, the mean moisture content was 24.2% and 23.1% for the edge and centre, respectively (Figure 4.3A). A similar pattern was found when soil PR was evaluated, showing a higher PR values on the inter-row compared to the under-vine (z=-8.52; p<0.001), although PR was higher in the centre than at the edge of the block (z=-3.56; p<0.001). No interaction between factors was observed (z=1.76; p=0.08). The mean PR value for the inter-row was 926.1 kPa and 1053.3 kPa for the edge and centre, respectively. Under the vines, the mean penetration pressure was 741.1 kPa and 769.6 kPa for edge and centre, respectively (Figure 4.3B).

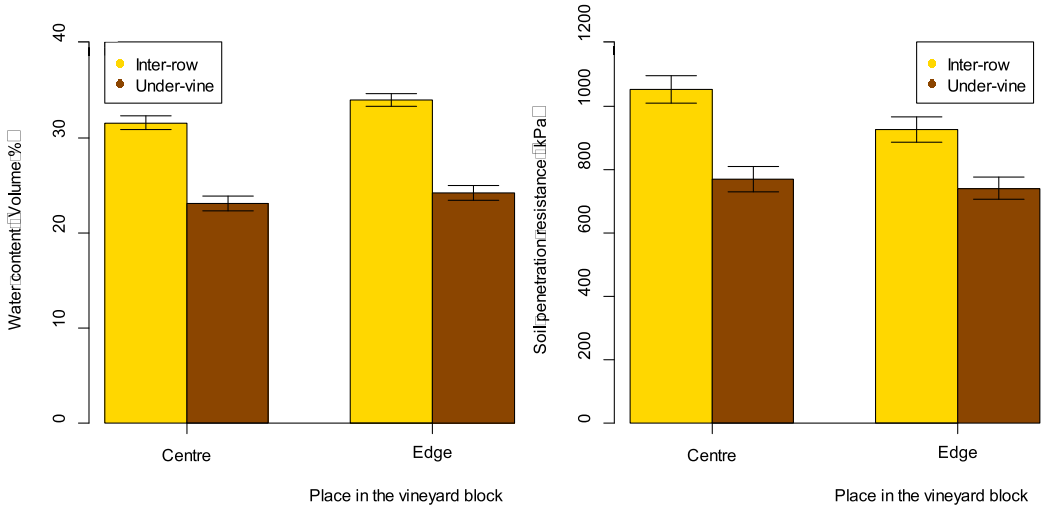

Figure 4.3. Soil condition measured in the under-vine and inter-row at the edge and centre of a vineyard at the Awatere Valley, during winter 2014. A) Moisture content (% volume). B) Penetration resistance (kPa). Confidence intervals (95%) are presented as error bars (two-standard errors).

4.4.3 Adult distribution within vineyard blocks

Adult density was higher on the edge of the vineyard block, compared to its centre (z=14.96; p<0.001). The mean number of adults per plant was 45.3 and 13.8, for the edge and centre, respectively. There was no significant differences between the two sampling dates evaluated (z=-1.89; p=0.06). When adult density was analysed along the vine row, the results differed between location and year. In 2014 at the Awatere Valley, adult numbers varied significantly along the row (t=-5.17; p<0.001). Also, the sampling date significantly explained the variation in abundance on the

three dates evaluated (χ^2=49.2; df=2; p<0.001), although the interaction between both was not statistically significant (χ^2=3.8; df=2; p=0.15) (Figure 4.4). For 2015 at the same location, the influence of the distance from the edge was not statistically significant to explain the variation in adult density by itself (t=0.08; p=0.93). However, the latter differed significantly amongst the three dates evaluated (χ^2=94.6; df=2; p<0.001). Also, the interaction between the distance from the edge and the sampling date was significant in explaining adult distribution along the vine row (χ^2=16; df=2; p<0.001), showing a change in distribution patterns from the edge towards the centre of the vineyard at the different sampled dates (Figure 4.5A). A similar trend was observed in Blenheim during 2015, in which the distance from the edge explained the variation in adult density along the vine row (t=-5.58; p<0.001), as well as between the different sampling dates (χ^2=116.3; df=2; p<0.001) and the interaction between them (χ^2=31.7; df=2; p<0.001) (Figure 4.5B).

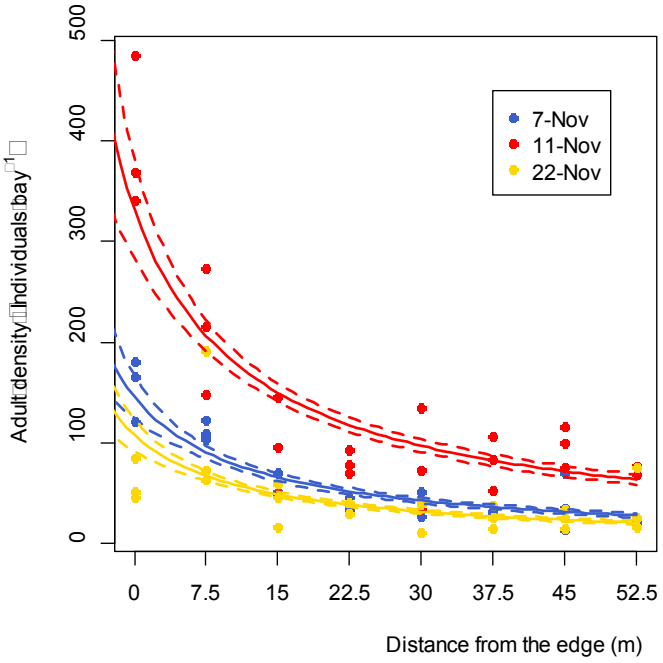

Figure 4.4. Adult density along the vine row (4 plants = one vineyard bay), from the edge towards the centre of the vineyard block, sampled during the 2014 flight season at the Awatere Valley. Regression curves were plotted using a generalised linear model (solid lines) with their confidence intervals at 95% (dashed lines). Three different sampled dates are shown, for the beginning, the middle and the end of the flight season.

4.4.4 Adult distribution through time

The seasonal abundance of adults that landed on vine plants is shown in Figure 3.1 (See Chapter 3). In 2014, a peak in adult abundance of 6,968 was found on November 14 at the Awatere Valley (Kono

Beverages), with a total of 36,369 adults collected during the flight season. In contrast, in 2015 the peak was only 1,039 on November 19, with a total of 6,111 collected adults. In Blenheim (Wither Hills), the peak in adult abundance was recorded on November 6, with 1,966 individuals. During the whole flight season, 14,731 adults were collected.

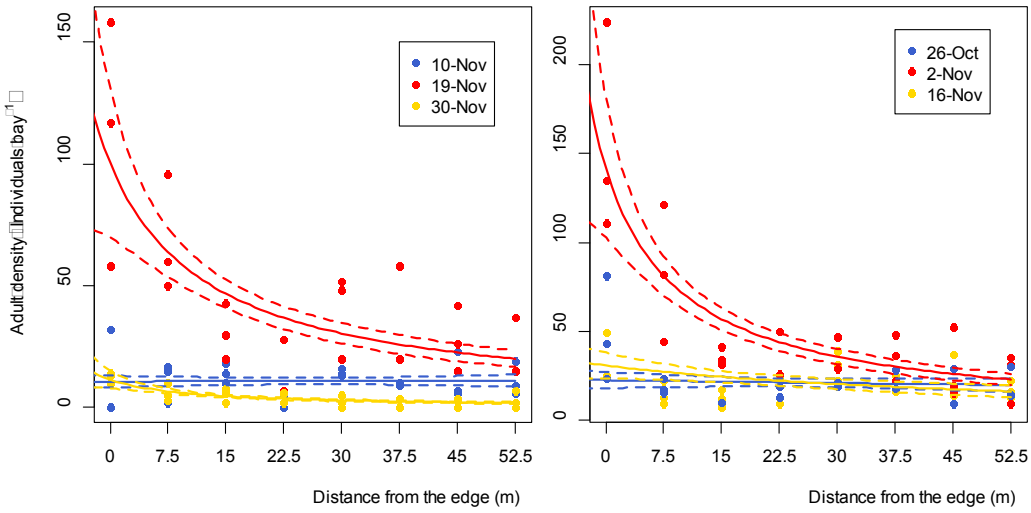

Figure. 4.5. Adult density along the vine row (4 plants = one vineyard bay), from the edge towards the centre of the vineyard block, sampled during the 2015 flight season. Awatere Valley (left) and Blenheim (right) sites are shown. Regression curves were plotted using a generalised linear model (solid lines) with their confidence intervals at 95% (dashed lines). Three different sampled dates are shown, for the beginning, the middle and the end of the flight season.

4.5 Discussion

Here, for the first time, the distribution of a melolonthid beetle is quantified in vineyards. Both adult and larval stages of *C. zealandica* were concentrated at the edge of the vineyard blocks, but larvae and adults were also present at the centre of the block. Larval and adult distribution was discussed in the context of soil conditions and female reproductive behaviour.

4.5.1 Larval distribution and soil condition

In the Awatere Valley, the mean of 103 larvae m^{-2} that was found under vines at the edge of the block, is lower than the 150 larvae m^{-2} found in dryland pastures and also lower than 200 larvae m^{-2} in irrigated pastures, previously proposed to be pasture density thresholds that produce severe economic damage in Canterbury, New Zealand (Townsend & Jackson, 1997). Nevertheless, a larval threshold to propose an economic injury level in vineyards has never been calculated, although (Mundy et al., 2005) suggested (using a pot experiment), that *C. zealandica* larvae feeding on vine roots, can only weaken young plants, but are unlikely to affect older plants. However, the effects of

larval densities at field level on the vine root system remains unknown. The differences found in soil MC and PR between under-vine and inter-row areas, at both edge and centre, could be related to viticultural management which relies heavily on machinery, increasing the compaction of the inter-row. Vehicle wheels do increase PR, affecting the MC in the soil profile (Hakansson et al., 1988; Horn et al., 1995). Similar results on vineyard soil compaction have been recently found in other Marlborough vineyards when weta (*Hemiandrus* sp. "promontorius", Orthoptera: Anostostomatidae) distribution was studied (Nboyine, Boyer, Saville, Smith, & Wratten, 2016). Considering that both measurements were performed in winter, it is likely that as compaction reduces soil hydraulic conductivity; soil evapotranspiration is also reduced, leading to higher values of MC in the inter-row compared to under-vine areas. Despite the statistical differences found in soil conditions, the ranges for MC (34%-31% for the inter-row and 23%-24% for the under-vine) did not affect *C. zealandica* larval mortality in pastures (van Toor & Stewart, 1986). Furthermore, under laboratory conditions a moisture range between 12% and 30% did not affect *C. zealandica* larval survival (Wightman, 1974b). In contrast, increased soil compaction has been identified as a strategy to reduce *C. zealandica* larval numbers in pastures by increasing the stocking rate (East & Pottinger, 1983) or by using a heavy roller pulled by a tractor (Stewart & Van Toor, 1986; van Toor & Stewart, 1986). The calculated pressure of a heavy roller (>6 t) on the soil surface to produce such a reduction in larval density is higher than 250 kPa (Stewart & Van Toor, 1986), although its effectiveness was also related to variations in soil moisture through the season (van Toor & Stewart, 1986). In terms of the PR values reported here, the difference between under-vine and inter-row for edge and centre was 185 kPa and 284 kPa, respectively. Therefore, the differences in larval numbers between under-vine and inter-row areas at the centre of the block could partly be influenced by soil compaction, but not at its edge. Despite the statistical differences reported here, larval numbers between 300-380 larvae m^{-2} have occurred in soils with PR ranges between 700 kPa and 1400 kPa, suggesting that the differences in the degree of compaction reported here are not enough to affect larval survival (East & Pottinger, 1975).

4.5.2 Larval distribution and adult behaviour

The distribution of *C. zealandica* has been described previously only in pasture ecosystems (Kelsey, 1951). In the 1970s, it was proposed that *C. zealandica* adults fly at dusk, searching for the highest plant silhouette available on which land (Farrell & Wightman, 1972). A recent study in Brazil showed that adults of *P. cuyabana* preferred to land on tall shrub patches, tall corn and soybean plants when compared to smaller ones, aggregating in the upper parts of those plants (García et al., 2003). Also, when trees were present in the middle of the crop, a higher abundance of *P. cuyabana* was found in the upper part of the trees (Oliveira & García, 2003). Similar adult preferences towards trees have been reported in Korean golf courses (Kim et al., 2013). In New Zealand vineyards, defoliation by *C.*

zealandica adults is initially concentrated (during the flight season) in the uppermost part of the vines (East et al., 1983). Those results could be related to a relict adult behaviour, as suggested previously by (Farrell & Wightman, 1972). From an evolutionary perspective, the New Zealand environment in which *C. zealandica* evolved comprised mainly shrubs and tussock grasslands (Merton, 1980). By landing above the grass layer, female pheromones could spread widely, increasing the likelihood of attracting males for reproduction, and therefore potentially enhancing the genetic pool of its offspring. In this context, flying males attracted to pheromones also land where females are present, contributing to its aggregated pattern. After mating, females lay eggs in the soil close to the mating sites, which leads to larval patches in pastures (Jackson & Klein, 2006; Kelsey, 1951).

The high proportion of larvae present in the under-vine areas at the edge of the vineyard block suggests that after adults land on vine plants for feeding and mating, they dropped to the ground (Blank, 1992; Farrell & Wightman, 1972). Eggs are then laid into the under-vine area, leading to differences in larval abundance between areas within the vineyard block reported here. This is in agreement with the higher proportion of adults collected at the edge of the vineyard block, when compared with its centre. In 2014, when adult distribution patterns along the vine row were analysed, a steady reduction in adult numbers was observed from the edge towards the centre of the vineyard block, irrespective of the sampling date. However, this pattern varied between the different sampled dates in 2015 at both studied locations. These differences in adult distribution along the vine row can be explained by inter-annual variations in adult abundance between the two studied flight seasons. Therefore, in high-adult abundance years during the flight season, higher adult density can be expected at the edge of the block compared to its centre, as the effects of the relict behaviour described above could be exacerbated by the high population of females in the area. Conversely, during low-adult abundance years, fewer insects land on the edge of the block, as the overall concentration of female pheromones could be dissipated in the air. This idea is supported by the adult density along vine rows in 2015, as more were found at the edge of the vineyard only at times of peak of abundance, while in the early and late stages of the flight season, the abundance of adults along the row did not differ. Unfortunately, the proportion of adults landing on the vine plants that originated within the vineyard block, and those that came from outside the vineyard remains unknown. Under this scenario, the origin of those invaders could be the grassy vineyard headlands, remnants of native vegetation, hedgerows and/or neighbouring vineyards. This pattern of invasion from outside the landing, feeding and mating sites has also been suggested for *Ectinohoplia rufipes* Motschulsky (Coleoptera: Scarabaeidae) (Kim et al., 2013) and *Melolontha melolontha* (Coleoptera: Scarabaeidae) (Wagenhoff et al., 2014). Recently, anecdotal evidence suggests that some Chilean

melolonthids present similar aggregation pattern at the edge of vineyards in Central and Southern-Central Chile (González-Chang, Personal observation).

4.5.3 Adult distribution through the flight season

Due to the wide range of biotic and abiotic variables involved in population dynamics, the reasons for a 6-fold difference in adult numbers between 2014 and 2015 are difficult to determine. However, it is likely that the severe drought that affected Marlborough area during the summer 2015 could have severely reduced larval populations, as low soil moisture has been previously identified as one of the soil parameters that affect larval survival and therefore, population dynamics on this species (East et al., 1981; Farrell, 1972). However, inter-annual variation in adult densities during the flight season is common in melolonthids, as reported for *M. melolontha* L. and *M. hippocastani* Fabricius in Europe (Wagenhoff et al., 2014).

4.5.4 Implications for pesticide reduction

The results discussed above have important implications for the control of this pest, which can lead to a reduction in pesticide applications. This can contribute to enhancing vineyard sustainability helping to maintain the good image and reputation that New Zealand wine industry has worldwide (Gabzdylova et al., 2009). The inter-annual variability suggested by a 6-fold reduction in adult abundance during the flight season between 2014 and 2015 highlights that the current prophylactic pesticide use may not be required every year, being necessary only during high-adult abundance years. The measurement of soil conditions, such as summer moisture content could be used as a proxy for larval survival, and therefore, adult density during the following flight season. Such an approach would require further research into the origin of flying adults and the development of a "larval sampling protocol" that can easily be deployed by the winegrowers. As larvae and adults are concentrated at the edge of the blocks, a key strategy to reduce pesticide use (if necessary) can be to limit its application to the edge of the vineyard blocks. This approach has been previously used against the carrot rust fly (*Psila rosae* L.) (Diptera: Psilidae), which lay eggs at the edge of carrot (*Daucus carota* L.) fields, achieving satisfactory levels of pest control by using only 10% of the recommended pesticide dose (kg ha^{-1}) (Sivasubramaniam et al., 1999). This localised approach to pest control has also been suggested for other pests that show an aggregated distribution at the edge of their crops, such as the brassica pod midge *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae) (Ferguson et al., 2003) and the cabbage seed weevil *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae) (Ferguson et al., 2000). Such strategy was also suggested for *D. virgifera*, which was concentrated on strips of corn used as an inter-crop in USA (Ellsbury et al., 1999).

In addition to localised pesticide application, habitat manipulation strategies can also be established at the edge of the block, such as the push-pull strategy (Cook, Khan, & Pickett, 2007; Frew et al., 2016; Khan, Pickett, van den Berg, Wadhams, & Woodcock, 2000). By placing a tall crop on the edge of the vineyard (i.e., on the vineyard headland), *C. zealandica* adults may land mainly there, reducing the damage to the vine foliage, shoots and inflorescences. Plant species that offer suitable traits to attract *C. zealandica* such as height and/or attracting volatile compounds, could be established in combination with species such as lucerne (*Medicago sativa* L.) which reduces larval survival in the soil by feeding-deterrent activity (Sutherland, Hood, & Hillier, 1975), or by direct toxicity (Sutherland, Hutchins, & Greenfield, 1982). The use of plant species that are attractive to adults in combination with those that can act as trap crops for larvae could form the basis of an agro-ecological strategy to reduce the damage caused by *C. zealandica* adults in vineyards, as suggested for other melolonthids attacking different crops (Frew et al., 2016; García et al., 2003).

4.6 Conclusions

In the present study, *C. zealandica* adults and larvae were concentrated in the edge of the studied vineyard blocks, with higher larval abundance in the under-vine areas when compared to the inter-row. Based on data presented here, it is strongly recommended that control strategies to reduce the damage caused by this endemic scarabeid in vineyards should be concentrated at the edge of the vineyard blocks. Furthermore, inter-annual variability in adult numbers based on the 6-fold difference found between 2014 and 2015 on the same site could indicate that pesticides are not needed every year, although no economic threshold or economic injury levels have been established for this pest in vines. Prophylactic use of pyrethroids is the main management practice to date in conventionally-managed New Zealand vineyards to control this pest, so the current work can help by limiting insecticide use to the edge of the vineyard blocks. Finally, this article highlights that by understanding pest distribution within agricultural crops, a localised strategy which concentrates pest control on high-pest density areas could reduce the use of pesticides. However, this requires population monitoring which farmers may not be trained or inclined to carry out.

Chapter 5

Mussel shell mulch can increase vineyard sustainability by changing *Costelytra zealandica* behaviour

5.1 Abstract

Global concern about external costs of pesticides (environment and human health) has promoted the development of new strategies for pest control in agro-ecosystems. Mineral-based dusts, such as hydrophobic particle films (HPF) and diatomaceous earths (DE) have been tested against several pests in different crops worldwide, highlighting their use as alternatives to synthetic pesticides. Also, optical barriers that alter insect visual orientation through UV manipulation have been used against flying insects. Recently, mussel shells (MS) optical properties have been investigated, suggesting that their UV-B reflective properties could affect insect behaviour, and therefore contribute as an alternative to orthodox pest control. In the present study, the effects of HPF, DE and MS were evaluated for their potential in reducing the endemic New Zealand scarab *Costelytra zealandica* damage in vineyards. HPF and DE significantly reduced beetles' damage by 46% when applied to Pinot Noir vines. MS treated vines had 69% fewer adults on them. When HPF and MS were combined, 33% and 73% reduction in damage was achieved by these two treatments, respectively. Furthermore, MS additions increased grape yield by 28%. The potential mechanisms behind these results are discussed in terms of insect behaviour and plant physiology. Although MS had a dramatic effect in reducing *C. zealandica* damage, and led to a subsequent grape yield increase, it also changed *C. zealandica* behaviour and distribution, with potential implications for scarab damage across the whole vineyard block.

Keywords: Feeding deterrents, sustainable pest control, seafood waste recycle.

5.2 Introduction

In recent years, increasing attention has been paid to the negative effects that synthetic-derived pesticides have on environmental quality (Tilman et al., 2001), pest resistance (Denholm, Devine, & Williamson, 2002) and human health (Stoytcheva, 2011). Despite the past success of these compounds for increasing crop yield (Tilman, 1999), recent advances in pest management science

have suggested the use of more ecologically-based approaches, including changing insect behaviour, as an important tool for sustainable pest management (Cook et al., 2007; Foster & Harris, 1997; Roitberg, 2007). Special interest has focussed on understanding the mechanisms behind behavioural changes that these inputs can have in terms of reducing pest damage (Roitberg, 2007). These changes usually involve alteration of insects' visual and/or olfactory perception of its environment (Cook et al., 2007; Foster & Harris, 1997). Although pest management has historically involved the use of olfactory behavioural disruptors, such as pheromones (Witzgall, Kirsch, & Cork, 2010), the application of different coloured reflective surfaces has also been studied to some extent to investigate behavioural changes mediated by the modification of insects' visual perception of its host (Prokopy & Owens, 1983). In aphids, reflective mulches placed on the ground have been used to reduce the damage caused by several species (González-Chang et al., 2017), with important consequences for the reduction of insect-borne viruses on a range of crops worldwide (Antignus, 2014). The mechanism behind this management relies on reflection of short-wavelength light (<400 nm) from those mulches, which might reduce the contrast between insects' host plants and their surrounding environment (González-Chang et al., 2017; Prokopy & Owens, 1983). Despite the beneficial effect of such mulches for pest control, the dramatic increase in the use of plastic reflective materials in agro-ecosystems during the last 10 years have raised major agronomic, economic and environmental concerns related to their removal and disposal (Kasirajan & Ngouajio, 2012). A potential environmentally-friendly alternative to this problem could partly involve the use of crushed mussel shells (MS). Studies in vineyards have shown that when MS are placed in the under-vine areas, short-wavelength light (UV-B, 280-320 nm) is reflected from the shells to the vine canopy (Crawford, 2007; Creasy & Ross, 2010). Therefore, it is plausible that the MS reflective properties might alter insect behaviour through an "optical barrier", as described above, where other reflective mulches were used to control several flying pests (Antignus, 2014). Although MS have been used before in filters (Zapater-Pereyra, Malloci, van Bruggen, & Lens, 2014) to enhance wine quality (Crawford, 2007; Creasy & Ross, 2010), and as a soil amendment (Álvarez, Fernández-Sanjurjo, Seco, & Núñez, 2012), they have never been tested as a control strategy to reduce pest damage in crops.

Feeding deterrents, such as hydrophobic particle films (HPF) and diatomaceous earths (DE) can also change insects' behaviour, offering a natural approach for pest control (Glenn et al., 1999; Korunic, 2013). The former is a product made of kaolin clays (kaolinite) bounded to a synthetic hydrocarbon that makes the clay hydrophobic (Glenn et al., 1999), and therefore less affected by rainy conditions when applied for pest control in the field (Glenn & Puterka, 2005). So far, different mechanisms have been discussed that alter insect behaviour; i) by producing a film on the treated plant foliage that produces a physical barrier between the insect and its host, therefore, reducing herbivores' ability to recognise their host plant; ii) by adsorption of kaolin particles around insects' body parts, affecting

movement and behaviour; and iii) by absorption of insects´ cuticles (Glenn et al., 1999; Glenn & Puterka, 2005). This approach has been used against several pest species on different crops over the last 15 years (e.g., Barker, Holaschke, Fulton, Evans, & Powell, 2007; Cadogan & Scharbach, 2005; Larentzaki, Shelton, & Plate, 2008; Lo Verde et al., 2011; Peng, Trumble, Munyaneza, & Liu, 2011), including coleopterans (Showler, 2002; Silva & Ramalho, 2013). On the other hand, DE are silica-derived particles from ancient diatom shells that settled in the bottom of the ocean after they died, between 20 and 80 million years ago (Korunic, 1998). When insects contact plant foliage treated with DE, damage reduction might also be achieved through behavioural changes involving the removal of diatoms from insects' cuticles (Ebeling, 1971; Korunic, 1998). However, the absorption of lipids present in insects' cuticles by these shells has been recognized as the main mechanism behind reduction in pest populations. This promotes water loss and therefore, insect desiccation (Ebeling, 1971; Korunic, 2013). The latter can be exacerbated by external injuries produced by the sharp edges that diatom shells can have (Korunic, 1998, 2013). In contrast to HPF, DE are easily removed from treated plants by rainy conditions, and for this reason, they have been extensively investigated in the area of stored agricultural products against several coleopteran species (Athanassiou et al., 2011; Kljajić et al., 2010; Korunic, 2013). However, DE have been recently used in combination with HPF to reduce vine cicada *Psalmocharias alhageos* Kolenati (Homoptera: Cicadidae) oviposition and nymph numbers (Valizadeh et al., 2013). This suggests that potential additive effects on pest control could be expected when HPF and DE are combined in field conditions.

In New Zealand vineyards, adults of the endemic beetle *Costelytra zealandica* White cause severe vine defoliation (New Zealand Winegrowers, 2013). At dusk, between October and November, *C. zealandica* adults' flight activity lasts for approximately 25 minutes (see Chapter 5), flying towards plant silhouettes to feed and mate (Farrell & Wightman, 1972). After a short period on the plants, they drop to the ground and lay eggs (Farrell & Wightman, 1972). As a consequence of this ephemeral presence on vine foliage, at both daily and monthly time scales, the prophylactic use of contact synthetic insecticides, such as pyrethroids, has dominated the vineyard management spectrum to control this pest since early 1990 (Blank, 1992). Considering adult *C. zealandica* flying and landing behaviour, in addition to the current non-sustainable reliance in synthetic pesticides, the use of HPF, DE and MS could offer an ecologically-friendly alternative to reduce adult grass grub damage in vineyards. Therefore, the aims of this work were i) to evaluate the contribution of HPF, DE and MS to reduction of damage caused by *C. zealandica* adults on vine plants, ii) to analyse the contribution of HPF and MS on grape yield, and ii) to quantify the effects of MS on adult *C. zealandica* behaviour.

5.3 Material and methods

5.3.1 Study sites

The Marlborough region in New Zealand is well recognized as an important area for wine production. Within that region, severe vine defoliation produced by *C. zealandica* adults has been recorded (New Zealand Winegrowers, 2013). Two Marlborough areas were selected to study alternative strategies to reduce adult *C. zealandica* damage in vineyards. In the Awatere Valley (41°44'S; 173°52'E) one vineyard block (2.21 ha; Kono Beverages; experiment 1) was used in spring 2014 to evaluate the effects of feeding deterrents and another to evaluate crushed mussel shells (6.12 ha; Kono Beverages, experiment 2). Such approaches were established and evaluated separately. However, during spring 2015, HPF and MS were combined in another vineyard block (4.58 ha; Wither Hills, experiment 3), close to Blenheim city (41°33'S; 173°55'E), to evaluate potential synergistic or additive effects. At both locations, organically-managed vineyards (cv. Pinot Noir) were used.

5.3.2 Experimental design

Experiment 1

In the Awatere Valley (2014), HPF (Surround WP©, USA), DE (Redox Pty Ltd, NZ), a mixture of both (HPF+DE) and a control were established in a randomized block design to test their efficacy to reduce adult *C. zealandica* damage. Each treatment was applied to five vines in each of two adjacent parallel rows (10 plants in total). Treatments were established along these two rows, next to each other. Unfortunately, separation between those was not possible along the row, due the spatial vineyard constraints. To counter this limitation to some extent, damage was measured only in the two central plants in each of those treated rows. Overall, 6 replicates were established separated by 5 m. The feeding deterrents were mixed with water and applied to the vine foliage using a 400 l hydraulic vine sprayer (Silvan G3, Australia), three times before the adult flight activity peak for 2014 occurred, on October 30, November 7 and 14 (See Chapter 3). In this experiment, the treatments were applied three times, due to the rapid foliage growth that occurred during that period. A dose of 20 g l^{-1} at a rate of 400 l ha^{-1} (8 kg a.i. ha^{-1}) was used (Valizadeh et al., 2013). When HPF and DE were combined, 10 g l^{-1} of each product was used to keep the 20 g l^{-1} concentration constant amongst treatments.

Experiment 2

To evaluate the effect of MS on adult grass grub numbers landing on vine plants, crushed MS were applied to the under-vine areas in the Awatere Valley (2014). This experiment was established at the edge of the vineyard block, as it has been suggested that *C. zealandica* adults aggregate in this area (see Chapter 4). For this reason, the first four plants along a vine row (comprising one bay) at the edge of the block were used. Treatments (MS and control) were established in a randomised block design, with 15 replicates. On each treated MS bay, 1.4 m^3 of previously-crushed MS were evenly

applied using a modified manure spreader (Millcreek, USA). This produced a 30 cm layer of MS on the under-vine area. These shells were crushed at Kono Seafood factory (Blenheim, NZ), as a strategy to reduce the volume of disposed shells after industrial mussel processing. *C. zealandica* adults were counted and then removed from treated and untreated rows between 21:30 h and 23:00 h on November 16, 2014.

Experiment 3

Based on the results obtained from the two experiments described above, the effect of MS and HPF on adult grass grub numbers, its damage on vine foliage and its effect on grape yield was investigated at Wither Hills, during spring 2015 and autumn 2016, respectively. HPF, MS, a combination of both (HPF+MS) and a control were established in a randomised block design (Figure 5.1A). HPF was selected over DE as feeding deterrent for this experiment due to its hydrophobic nature (Glenn et al., 1999), but also because no statistical differences were found between HPF and DE on adult damage (see results). HPF was applied using a 20 g l^{-1} concentration at a rate of 400 l ha^{-1} only once, on November 1, 6 days before the adult flight peak of abundance for the 2015 season was recorded (see Chapter 3). All treatments were established at the edge of the vineyard block, using the first bay (a group of four vine plants along the row) from the block edge towards its centre (Figure 5.1A). On each MS or HPF+MS treatment, crushed shells were applied as described for experiment 2. Eight replicates were used separated by 10 m.

5.3.3 Adult *C. zealandica* activity

At Wither Hills (2015), beetle activity was recorded on vines with and without MS using the arrangement of experiment 3 (Figure 5.1). Adult activity was measured using security surveillance infra-red sensitive video cameras (Sanyo VCC-HD4600P, China). Two cameras were established at 50 cm in front of the first vine plant in a row; one on a MS-treated bay and the other on control (Figure 5.1C). Cameras were established at 1.5 m over the ground using a tripod. Beetle activity in those two plants was recorded on five consecutive days, starting on November 6 until November 10, from 19:00 h to 23:00 h. On each date, all videos were recorded using 1280 x 720 pixel resolution and encoded afterwards into MKV format (Matroska Video Codec) using VSDC free video editor software (Flash-Integro LLC, v.4.0.1.479). Each video was subsequently cut from the start to the end of adults' flight activity. Then, screenshots of those edited videos were taken every minute using VSDC software. On each screenshot, every white dot found was counted, as well as "shadow" dots that appeared on the plants. Each dot (shadow or white) represented one *C. zealandica* adult. These shadows were produced by adults flying in front of the infra-red light, projected over the white background produced by the vine plants. Due the lack of image background contrast produced by the infra-red light over the vines, all white and shadow dots in the screenshots were counted manually.

Through the analysis of the screenshots, two different behaviours were recognised; i) hovering or flying, in which each counted dot was over the vines, changing its position between two consecutive screenshots, and ii) landing, where each counted dot was on the vines, without changing its position. The presence/absence of dots between screenshots was assessed by rapidly alternating between two consecutive screenshots in the computer screen, allowing for the identification of flying/hovering and/or landed beetles. These white dots and shadows were counted using the "cell counter" function within the software Fiji (Schindelin et al., 2012). A similar approach that compares changes in animal presence/absence between two photographs has been recently used to study salmon (*Oncorhynchus* spp.) passage and abundance in Alaskan streams (Deacy, Leacock, Eby, & Stanford, 2016).

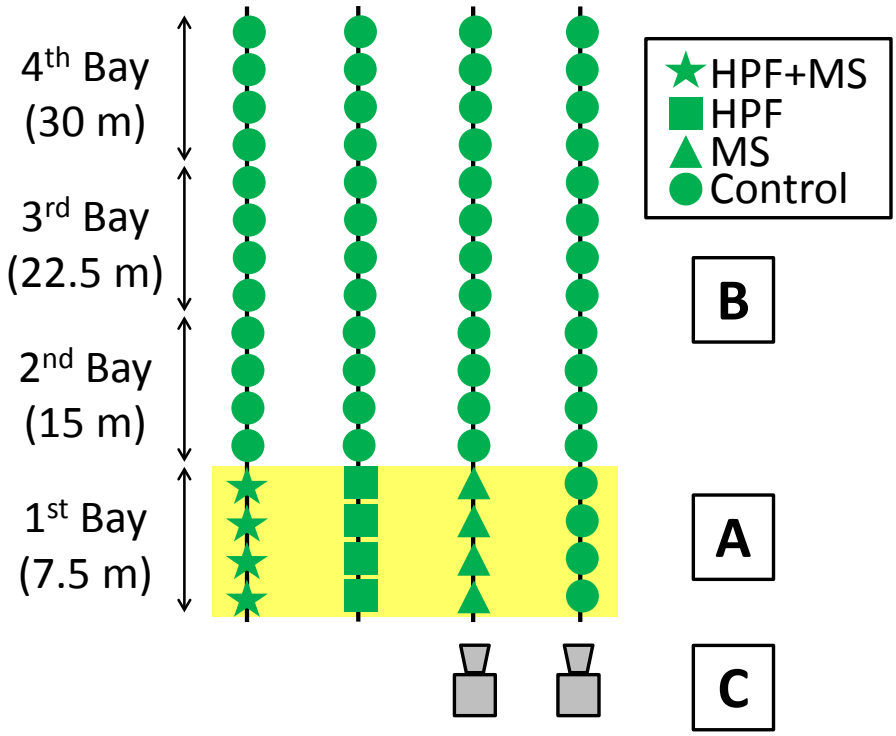

Figure 5.1. Experimental design used for three different experiments (A, B, C). Symbols represent one vine plant along a vine row. A) Random distribution (of one replicate) of mussel shell mulch (MS, triangle), hydrophobic particle films (HPF, square), both combined (HPF+MS, star) and control (circle) on leaf damage and grape yield, applied at the end of the vine rows in Wither Hills. The distance from the edge of the block towards its centre is shown in brackets. B) Second, third and fourth bay used to evaluate adult *C. zealandica* distribution when experiment "A" was present in the first bay. Adult counts were taken from all 4 bays. "B" represent one replicate. C) Position of two infra-red video cameras at the ends of two treated vine rows (MS and control) to study adult beetle activity. Data were recorded for 5 consecutive days. Each date was considered a replicate.

5.3.4 Plant damage and grape yield

Adult *C. zealandica* damage on vine leaves starts from their margin towards their centre, leaving the leaf veins untouched when severe cases of defoliation occur (González-Chang, Personal observation). Similar damage patterns have been recorded on tamarillos (*Solanum betaceum* Cav.) (Blank & Olson, 1982). Damaged leaves (DL) were identified and counted on each vine plant considered in experiment 1 and 3. Damage was expressed as the number of DL on the total number of leaves (TL) per plant. Damage assessment was performed at the end of the adults' flight season, in early December 2014 and 2015. Grape yield was measured as weight of all grape bunches per plant (kg plant^{-1}) only in experiment 3. An electronic portable balance was use for the latter (CAS SW series, Korea).

5.3.5 Adult *C. zealandica* vineyard distribution after mussel shells application

The same randomised block design applied to experiment 3, was used to investigate the potential effect of those treatments applied at the edge of the vineyard block on adult *C. zealandica* distribution (Figure 5.1B). Therefore, adults were collected on the first, second, third and fourth bay along the vine row (Figure 5.1B). In those selected bays, adults were counted on the vine foliage, and then removed by hand between 21:30 h and 23:00 h on November 16, 2015.

5.3.6 Statistical analyses

Data from experiment 1 and 3 (damage and yield) were pooled and averaged on each replicate within their respective treatments to correct spatial pseudoreplication (Crawley, 2007). Proportional data were corrected using a square root arc-sine transformation to fulfil the necessary requirements for an analysis of variance (ANOVA). Yield data was homoscedastic and normally distributed, therefore ANOVA was carried out. A post-hoc analysis was performed using Tukey's honest significant difference test, using the R package "Multcomp" (Hothorn et al., 2016). To evaluate the effect of MS on the number of adults that landed on vines in 2014 (experiment 2), data were $\log_{10}$ transformed, and subsequently, a paired t-test was carried out between MS-treated bays and control. The same analysis and transformation were performed to evaluate adult numbers on MS, HPF, and HPF+MS treated vines (experiment 3).

Mean adult beetle activity data (beetle min^{-1}) from the screenshot analysis was calculated for 5-min time periods. During the flight period recorded, 6 periods were created at 5, 10, 15, 20, 25 and 30 min after adults began to fly. Such periods included data on mean beetle activity (flying/landing) during that period. Due to a lack of homoscedasticity in the data, they were transformed using a spread-stabilizing power transformation with the R package "Car" (Fox et al., 2016). No autocorrelation was found between data from the five different recorded dates, so these were

considered as replicates. Each period was analysed independently, using a paired t-test between MS and control treatments. Adult distribution from the edge towards the centre of the vineyard block after HPF and MS were applied was analysed using generalised linear mixed effect models (GLMMs). In these, the interaction between the treatments (MS, HPF, HPF+MS and control) and the position in the row in which adults were collected (1^{st}, 2^{nd}, 3^{rd}, 4^{th} bay), were considered as fixed effects, while the row in which those bays occurred was treated as a nested random effect (bays within a row) (Figure 5.1B). A negative binomial error family was used to correct overdispersion (Zuur et al., 2009). A Wald test with a χ^2 distribution was used to assess the significance of the treatments and the position in where beetle samples were taken (B. M. Bolker et al., 2009). GLMMs were performed using the R packages "MASS" (Ripley et al., 2016), "Matrix" (Bates & Maechler, 2016), "Lme4" (Bates et al., 2016) and "Aod" (Lesnoff & Lancelot, 2015). All statistical analyses were calculated using the software R v.3.2.5 (R Core Team, 2016).

5.4 Results

5.4.1 Adults landing on vines

The addition of MS to the under-vine areas significantly reduced the number of adult *C. zealandica* that landed on those vines by 69% (t=-9.46; df=14; p<0.001). In control plots, the mean number of adults was 189 adults bay^{-1}, while on MS-treated vines these were 59 adults bay^{-1}. In 2015, adult numbers on MS (t=3.32; df=7; p<0.05) and HPF+MS (t=5.26; df=7; p<0.01) treated vines were statistically fewer than control. However, non-significant differences were found between HPF and control (t=0.37; df=7; p=0.71), nor between MS and HPF+MS (t=2.28; df=7; p=0.06). The mean number of adults per bay was 56.5, 54, 27.5 and 16, for control, HPF, MS, and HPF+MS, respectively.

5.4.2 Plant damage and grape yield

During 2014, feeding deterrents (HPF and DE) statistically reduced damage caused by adult *C. zealandica* compared to control ($F_{3,20}$=7.98; p<0.01). No differences were found between the feeding deterrent treatments used (p>0.8) (Figure 5.2). In this experiment, the application of these deterrents on Pinot Noir vines reduced damage by 46%. In 2015, when HPF and MS were evaluated, a significant damage reduction was also found ($F_{3,28}$=31.9; p<0.001). A 33% and 73% damage reduction was achieved by HPF and MS, respectively (Figure 5.3). The combination of both approaches (HPF+MS) was not statistically different from the only-MS application (p=0.9). Grape yield was significantly increased ($F_{3,28}$=3.12; p<0.05) from 3 to 4.2 kg $plant^{-1}$ when MS were present. No differences were found when HPF was applied alone (p=0.5), nor between MS and HPF+MS (p=0.9) on grape yield. This addition of MS led to a 28% increase in grape yield (Figure 5.4).

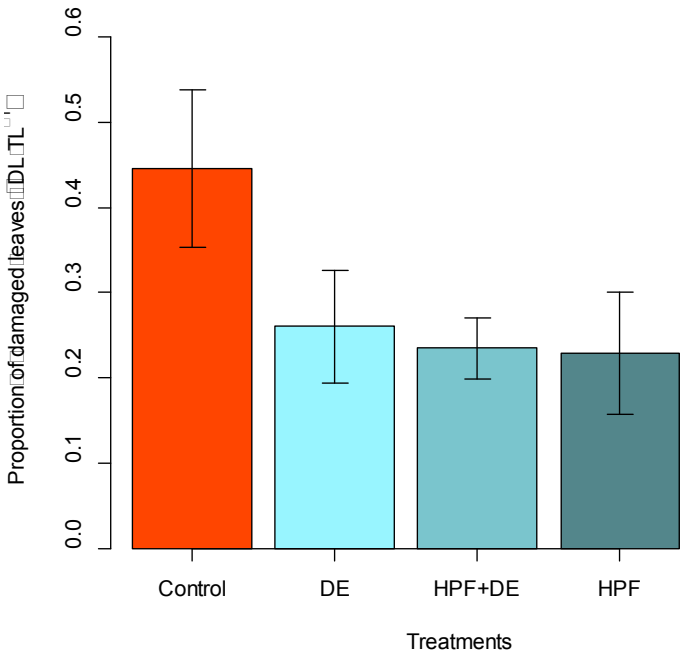

Figure 5.2. Proportion of damaged vine leaves (DL TL^{-1}) under different feeding deterrents. No statistical differences were found between diatomaceous earths (DE), hydrophobic particle films (HPF), and both approaches combined (HPF+DE) ($p>0.8$). All treatments were significantly different from control ($F_{3,20}=7.98$; $p<0.01$). Error bars represent two-standard errors.

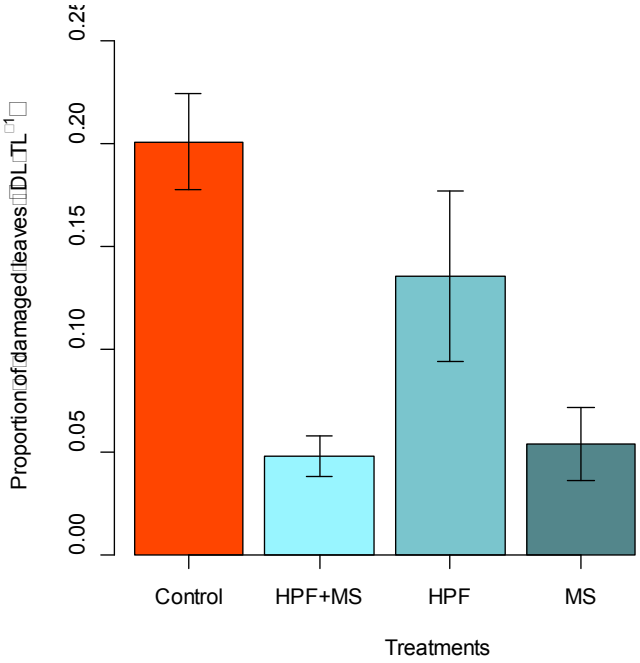

Figure 5.3. Proportion of damaged vine leaves (DL TL^{-1}) under hydrophobic particle films (HPF), mussel shells (MS) and both combined (HPF+MS). All treatments were statistically different from control ($F_{3,28}=31.9$; $p<0.001$). No differences were found between HPF+MS and MS ($p=0.9$). Error bars are two-standard errors.

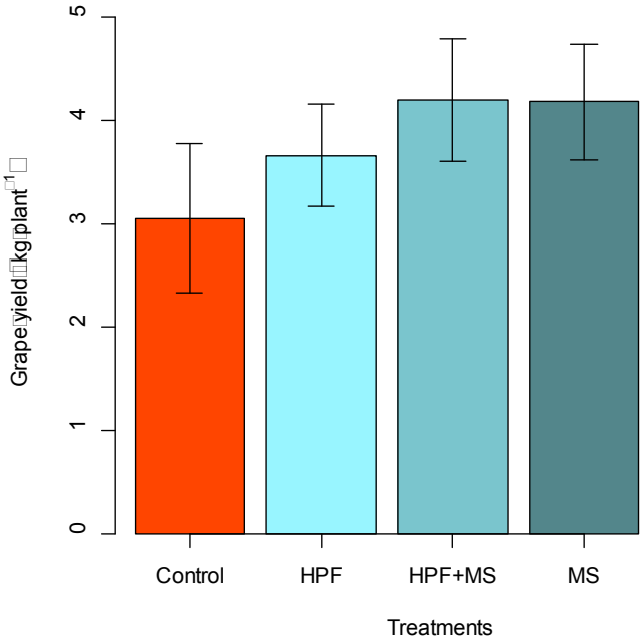

Figure 5.4. Effects of hydrophobic particle films (HPF), mussel shells (MS) and both combined (HPF+MS) on grape yield (kg plant^{-1}). HPF+MS- and MS-treated vines were statistically significant from control ($F_{3,28}$=3.12; p<0.05). HPF was not different from control (p=0.5). Error bars are two-standard errors.

5.4.3 Video analysis of adult behaviour

Adult *C. zealandica* began its flight 20 minutes after sunset, from 20:31 h on November 6th, until 20:39 h on November 10th. This behaviour stopped between 20:57 h and 21:06 h over the studied dates, respectively, with a mean flight time of 26 minutes each day. Adult hovering activity (beetles min^{-1}) was lower over MS treated plants, 10 (t=3.49; df=4; p<0.05) and 15 min (t=3.29; df=4; p<0.05) after adult flight activity started (Figure 5.5). Landing activity was also lower in the MS treatment, 10 (t=2.76; df=4; p=0.051), 15 (t=5.22; df=4; p<0.01), 20 (t=4.51; df=4; p<0.05), and 25 min (t=4.4; df=3; p<0.05) after adult flight activity started (Figure 5.6).

5.4.4 Adult distribution within vineyard blocks

The number of adults that landed along the vine rows was significantly affected by the treatments applied (χ^2=42.7; df=3; p<0.001). In addition, there was a significant interaction between treatments and the number of adults collected in the different bays along the vine rows (χ^2=40.2; df=3; p<0.001). When MS treatments were present (MS and HPF+MS), the mean number of adults was 21.7 and 44 adults bay^{-1}, in the 1st and 4th bay, respectively. Conversely, in the control and HPF treatments the

mean number of adults was 55.3 and 29.8 adults bay^{-1}, in the 1st and 4th bays, respectively (Figure 5.7).

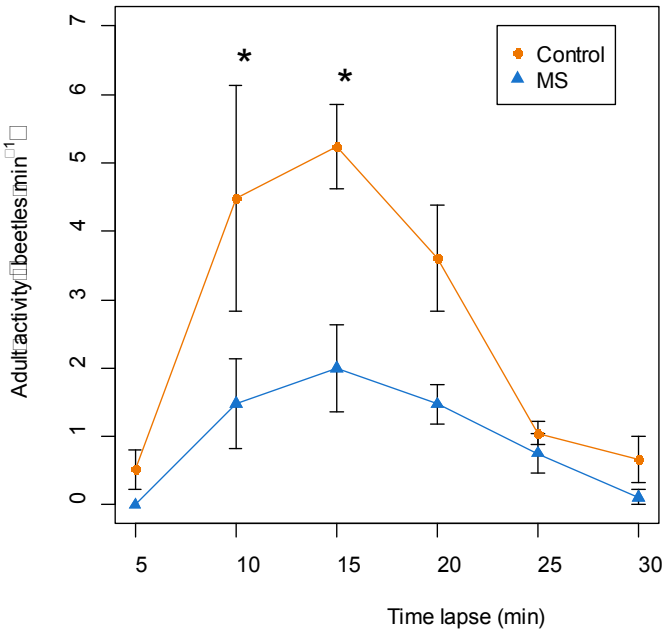

Figure 5.5. Flying/hovering adult *C. zealandica* activity on un-treated (upper line) and MS-treated vine plants (bottom line). Error bars are two-standard errors. Significant differences between treatments at each time interval are presented in the figure (* p<0.05).

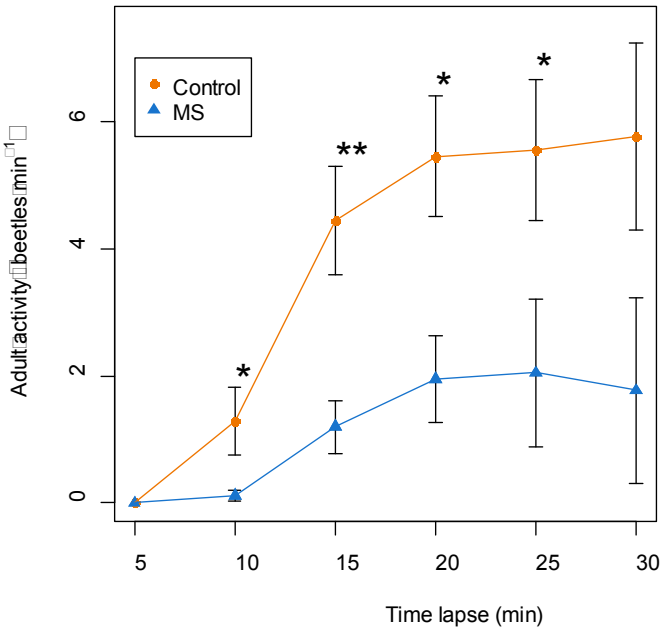

Figure 5.6. Adult *C. zealandica* landing activity presented on un-treated (upper line) and MS-treated vines (bottom line). Error bars are two-standard errors. Statistical differences between treatments at each time interval are presented in the figure (* p<0.05; ** p<0.01).

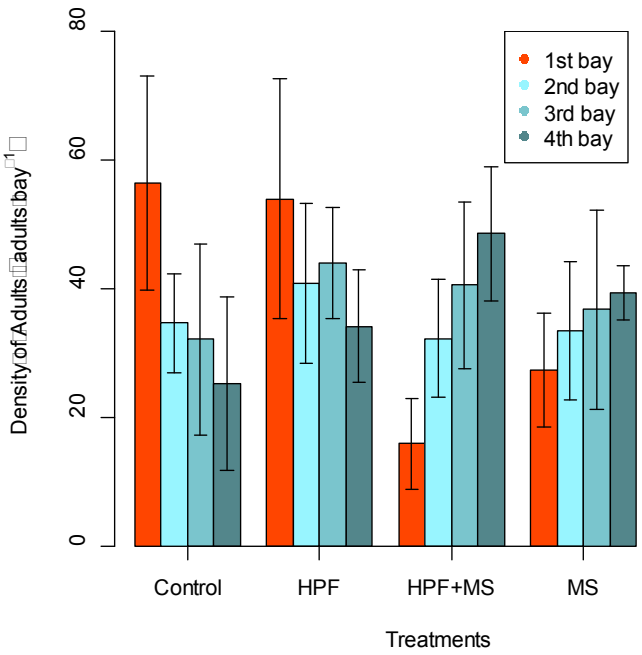

Figure 5.7. Adult *C. zealandica* distribution along vine rows, from the end of the rows towards the centre of a vineyard block. Different bars within hydrophobic particle films (HPF), mussel shells (MS), and both treatments combined (HPF+MS), represent the different bays along the vine row (a group of 4 vine plants), where adults were collected. On each treatment, from the left to the right, the first, second, third, and fourth bays are presented. The interaction between those treatments and the bay's position along the row was significant (χ^2=40.2; df=3; p<0.001). Error bars are two-standard errors.

5.5 Discussion

This work presents for the first time the effect of crushed mussel shells (MS) applied to under-vine areas on insect pest behaviour. In contrast, hydrophobic particle films (HPF) and diatomaceous earths (DE) have been used extensively in the past for pest control (Athanassiou et al., 2011; Barker et al., 2007; Glenn & Puterka, 2005; Kljajić et al., 2010; Korunic, 2013; Lo Verde et al., 2011; Silva & Ramalho, 2013), with described mechanisms behind their mode of action. For this reason, this discussion is focused on but not restricted to the potential unexplored mechanisms involving the UV-B reflective properties of MS (Crawford, 2007; Creasy & Ross, 2010) on pest control and grape yield.

5.5.1 Beetle behaviour and mussel shells application

Host location by insects is strongly influenced by olfactory and visual stimuli (Cook et al., 2007; Foster & Harris, 1997). Considering the ubiquitous nature of the organic volatile compounds emanating from the crushed MS in these experiments (which could be perceived some kilometres away from

the plots), it is likely that such volatiles were also present on the control treatments. This suggests that a potential mechanism behind the differences in adult numbers between MS and control treatments was associated with changes in the visual perception of the plant host by adult *C. zealandica* instead. Short-wavelength UV-B radiation emission from MS has been quantified before in New Zealand (Crawford, 2007; Creasy & Ross, 2010) and USA (Sandler, Brock II, & Vanden Heuvel, 2009). Because these short wavelengths play a major role in insect orientation, navigation, feeding and mating (Dacke, Nilsson, Scholtz, Byrne, & Warrant, 2003; Seliger, Lall, & Biggley, 1994), the use of different materials that manipulate UV-B reflection altered the behaviour in *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) (Nguyen, Borgemeister, Max, & Poehling, 2009), *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Antignus, Nestel, Cohen, & Lapidot, 2001), *Myzus persicae* Sulzer (Homoptera: Aphididae) (Chyzik, Dobrinin, & Antignus, 2003), and *Frankliniella occidentallis* (Thysanoptera: Thripidae) (Kigathi & Poehling, 2012). This may explain the significant reduction of the flying/hovering activity of *C. zealandica* around MS-treated vines and therefore, the reduced number of individuals landing on those. It has recently been suggested that *C. zealandica* adults aggregate at the edge of vineyard blocks due the expression of a relict adult behaviour (see Chapter 4). Female pheromones released after landing on tall plants might increase the likelihood of attracting males from surrounding areas, which has potential evolutionary consequences for increasing the genetic diversity of its offspring (Merton, 1980). This could explain the hovering behaviour of males over vines, reflecting their attempts to locate the females that have already landed on those. Recent unpublished data support the previous idea, as sex-ratio analysis has indicated that a higher proportion of females on the vines were found after *C. zealandica* adults were successively removed from the vines every five minutes after flight activity started (See Chapter 3). Conversely, under natural conditions sex-ratio was male biased at the beginning of the flight (Farrell & Wightman, 1972).

In a previous non-manipulative study, adult *C. zealandica* numbers were higher at the ends of the vine rows (1st bay), decreasing gradually towards the centre (8th bay) (see Chapter 4). This work showed that MS application at the edge of the vineyard block reduced the number of adults there but increased its numbers towards the centre (Figure 5.7). This change on beetles' distributional patterns might have implications for larval densities and adult damage distribution within vineyard blocks, therefore this behavioural change needs further investigation. Attention should focus on the number of bays (or rows) that need to be treated in order to deter adults from landing further into the block.

5.5.2 Damage reduction and grape yield increase

In vines, HPF and DE have previously been combined to control the vine cicada *P. alhageos*, producing a significant reduction on its egg numbers by 55%, and subsequently, an increase on grape yield by 48% (Valizadeh et al., 2013). The latter was attributed to the hydrophobic barrier produced by HPFs on the leaf surface, which limits the ability of *P. alhageos* to recognise its host, reducing the amount of eggs laid by this pest. However, in the present study, no significant differences were found when HPF and DE were mixed compared with solely the HPF or DE application, suggesting that the presence of this hydrophobic barrier might not be the mechanism behind *C. zealandica* damage reduction, as DE do not produce such a barrier (Korunic, 2013). Previous studies have suggested that HPF deterrency might also be based on altering insect visual perception of its host plant, reducing the numbers that land on those (Glenn & Puterka, 2005; Showler, 2002; Silva & Ramalho, 2013). However, in this work, the number of adult *C. zealandica* on HPF-treated plants was not different from control. Considering that both HPF and DE particles attach to insect's body parts (Ebeling, 1971; Glenn & Puterka, 2005), *C. zealandica* adults might attempt to remove them, reducing their movement on vine leaves (Glenn et al., 1999). Also, plant palatability could be decreased by the presence of silicon-based particles (Massey, Ennos, & Hartley, 2006). Furthermore, it has been suggested that HPF can obstruct insects´ digestive system (Showler, 2002). It is therefore likely that a combination of these factors altered adult *C. zealandica* feeding behaviour after landed on vines.

Reflective mulches, such as plastic, aluminium, and geotextile membranes have been tested to improve wine quality and increase grape yield (Hostetler, Merwin, Brown, & Padilla-Zakour, 2007a, 2007b; Osrečak, Karoglan, & Kozina, 2016; Sandler et al., 2009). Also, increases in grape yield through the addition of MS to the under-vine row areas have been reported before. In the USA, MS-treated vines (cv. Chancellor) produced 5.56 kg plant^{-1} compared to 4.21 kg plant^{-1} in control plots, which is a 32% increase (Sandler et al., 2009). However, when MS-treated vines (cv. Pinot Noir) were evaluated in New Zealand no-significant differences were observed in grape yield (Crawford, 2007; Creasy & Ross, 2010). This differential response in grape yield might be related to physiological differences between those cultivars under conditions of environmental changes, as described before between Shiraz and Grenache varieties (Soar et al., 2006). Additionally, mollusc species used in those experiments varied, as *Mercenaria mercenaria* L. (Veneridae) was used in the USA, while *Perna canaliculus* Gmelin (Mytilidae) was the species in New Zealand. Is likely that the different shell pigmentation between these two species affected the amount of reflected photosynthetically active radiation into the canopy (Creasy & Ross, 2010; Sandler et al., 2009). This is in agreement with significant grape yield differences found in the USA (cv. Pinot Noir) when black geotextile membranes yielded 1.38 kg plant^{-1} compared to 2.23 kg plant^{-1} under white geotextile membranes (Hostetler et al., 2007b). In this work, the 28% increase in grape yield could be the expression of three different

factors acting together, namely i) insect damage reduction, ii) UV-B effects on plant physiology, and iii) soil moisture increase. The 73% reduction in vine defoliation attributed to the "optical barrier" produced by MS UV-B reflective properties on *C. zealandica* behaviour, probably increased vine-plant leaf area, which might have enhanced the photosynthetic rate (Petrie, Trought, Howell, & Buchan, 2003). Also, UV-B light can regulate the guard cells in leaf stomata, irreversibly closing or opening them (Jansen & van den Noort, 2000). This permanent stomata opening might increase CO_2 absorption from the surrounding atmosphere, enhancing the overall amount of carbohydrates produced through photosynthesis (Roelfsema & Hedrich, 2005). In addition, UV-B has been associated with the jasmonate pathway (Ballaré, 2014), which plays a major role in plant induced defences against herbivorous insects (Howe & Jander, 2008). Finally, the increased soil moisture previously found under MS-treated vines (Crawford, 2007; Creasy & Ross, 2010; Sandler et al., 2009) could increase the amount of water available to the fruits, and thus enhance grape yield (Reynolds & Naylor, 1994). A better understanding of the mechanisms behind the reflective and other properties of MS on insect behaviour and plant physiology could replace the use of plastic-derived mulches, especially considering the current high cost of biodegradable plastic mulches (Kasirajan & Ngouajio, 2012). Similar economic constraints were reported when geotextile membrane mulches were used to enhance wine quality and grape yield (Hostetler et al., 2007a, 2007b). Conversely, MS are inexpensive and easily available (where mussel farms are close) and if not recycled they are disposed in local landfills, with eventual ecological and social consequences (Iribarren, Moreira, & Feijoo, 2010). In this context, MS mulches might have a role as alternatives to enhance vineyard sustainability, and also have potential application for other horticultural crops that today rely heavily on plastic mulches for weed and pest control, moisture retention, crop yield increase and food quality.

5.6 Conclusions

The results presented here suggest that HPF, DE, and MS can be used as a sustainable strategy to reduce the damage caused by *C. zealandica* adults in vineyards. Furthermore, MS application increased grape yield by 28%, which was explained by behavioural changes in flying (hovering) and landing patterns exhibited by this pest. Although the mechanisms behind this behavioural change and grape yield increase were not evaluated here, it was suggested that MS reflective properties in the UV-B spectrum range might be responsible for these two phenomena. The use of crushed MS for pest control has never been reported before, so the results presented here open a new opportunity to recycle these shells, which otherwise will be disposed of in the local landfill. However, the effects of this treatment on insect behaviour need further investigation, as MS deterrent effects on *C.*

zealandica adult landing behaviour might lead to higher infestation rates in other parts of the vineyard if placed only at its edge.

Chapter 6

Discussion

The human race faces problems that have never been present in its history before. Environmental problems, such as biodiversity loss, land degradation and climate change threatens an endless number of life forms that are intimately entwined with human development, culture and evolution (Rockström et al., 2009; Steffen et al., 2015). Unfortunately, despite the urgent need for a change in human behaviour, this seems to be trapped by political and economic interests, diluted thereafter by the un-informed media. An example of the previous statements lies in modern agriculture, which largely contributes to the environmental problems mentioned before (Reganold & Wachter, 2016; Turner et al., 2016). The latter is due its indiscriminate use of fossil-fuel based inputs and the need for large monocultural areas (Tilman et al., 2001). Although more than 235 thousand scientific articles were published from 1960 until 2015 in relation to sustainable agriculture (Figure 1.1), modern agriculture still contributes in a major way to environmental degradation and socially-related problems (Reganold & Wachter, 2016). Probably, one of the reasons for this unreasonable lack of adoption of this vast amount of scientific research is that there is still a gap on how research performed by universities and research institutes is transferred to farmers, which is accentuated by the lack of locally based "recipes" to apply that knowledge at farm level (Gurr et al., 2017). In this process, the participation of farmers is crucial (Altieri, Funes-Monzote, & Petersen, 2011; Méndez et al., 2013; Warner, 2008) in order to highlight their agricultural constraints, which should guide to some extent the development of applied agro-ecological sciences (Aeberhard & Rist, 2009; Méndez et al., 2013; Wezel et al., 2009). An example of the latter is the research presented throughout this document. This PhD thesis aimed to reduce the damage caused by adults of the endemic New Zealand grass grub, *Costelytra zealandica* White, without using synthetic pesticides. This issue was previously highlighted by New Zealand's organic winegrowers, as before this thesis, no work was done in order to control this pest in an environmentally-sound way (Scarratt, 2011).

The approach used here to help winegrowers to reduce the pesticide load on their vineyards was a combination of field and laboratory work, the latter comprising the development of an improved rearing protocol to enhance the survival rate of *C. zealandica* adults from field-collected larvae (Chapter 2). The field component comprises non-manipulated adult and larvae distribution within vineyard blocks (Chapter 4), and the analysis of this insect's sex-ratio throughout its flight season (Chapter 3). Experimental work involved the removal of adult beetles after landing on the vine foliage to assess its landing dynamics (Chapter 3) and the use of alternative natural products, such as

hydrophobic particle films (Glenn et al., 1999), diatomaceous earths (Korunic, 1998), and crushed mussel shells to reduce the damage caused by this insect pest (Chapter 5). The understanding of adult *C. zealandica* behaviour on vines (Chapters 3 and 4), contributed to the evaluation of the different management strategies described in Chapter 5. Notwithstanding the constraints that a limited 3-year PhD programme has, a final sustainable-management protocol will be delivered to New Zealand winegrowers after some additional experiments that will address some important unanswered questions that arose during this intellectual endeavour. Due to timing and financial constraints, these are not presented as experimental chapters here, but highlighted as future research avenues in section 6.1.4 below.

6.1 Overall outcomes of the study

6.1.1 Adult *C. zealandica* rearing protocol

Before this PhD work, few publications have reared adults from field-collected larvae of this species due the low survival rates achievable (Lefort et al., 2015; Wigley & Dhana, 1992). After increasing the survival rate from larvae to adults, from 25 to 83% by using a closed container, instead of the ice-cube trays used before for this purpose (Wigley & Dhana, 1992), this new rearing approach will certainly encourage researchers interested in studying aspects of Melolonthinae biology and behaviour in New Zealand, and eventually around the world. Despite the lack of proper replication in the treatments used in Chapter 2, due the differential larval weight gain under each treatment, the dramatic difference between the treatments, revealed after the application of a Chi-squared test of independency, highlights the efficacy of the protocol proposed here and opens the possibility to discover its future applicability.

Adults obtained from the rearing method described in Chapter 2 were used in feeding choice-tests to assess adult *C. zealandica* feeding preferences. Previous work successfully performed feeding tests with adults collected from the field (i.e., Farrell , 1975; Wigley & Dhana, 1992), or with adults reared from field collected pupae (i.e., Farrell 1973). However, feeding tests for this endemic scarab species have never been done before with adults reared from field collected larvae. By applying a basic choice-test briefly described below, insights into plant selection for a potential “push-pull” strategy can be empirically tested under laboratory conditions. For this reason, 10 pairs of adults (10 males and 10 females) reared from field collected larvae (Chapter 2), were placed in a plastic box (4,5 L), filled to a half of its height with 20% w/w moist soil from Lincoln University’s Field Service Centre arable land. In each plastic box, 3 leaves from *V. vinifera* and 20 from *T. repens* were left in the soil surface. The leaves from each species were left at opposite sites within the box, and adults were

released in the centre of the box. Then, the boxes were covered with muslin fabric, and distributed based on a randomised block design inside a climate-controlled room for one week at 20°C with a 16:8 light-dark photoperiod. Unfortunately, feeding did not occur in the experiment described above. The lack of feeding in *C. zealandica* adults reared from field collected larvae has never been reported before. This unexpected result suggests that some environmental factors might be triggering the feeding behaviour in *C. zealandica* adults. Interestingly, environmental parameters used here such as, soil moisture, temperature and photoperiod were similar to the ones previously applied by Farrell, (1975). Future work should address if the lack of adult feeding found here (if consistent with future work) is related to: i) the food type provided to the larvae for their development, ii) the potential emission of volatile compounds from the cut vine leaves that might have interrupted adults' feeding behaviour, iii) or simply a laboratory artefact in the rearing method (Chapter 2) and/or in the choice test performed here.

6.1.2 Sex ratio analyses through the *C. zealandica* flight activity period

Female *C. zealandica* arriving at vines before males do has interesting potential applications for pest control. Despite the inherent value of this knowledge in its own right, it might contribute to further develop a management strategy to control this pest, such as with *Melolontha melolontha* L. and *M. hippocastani* F., where aspects of those species' chemical communication were explored to develop an optimised lure (Reinecke et al., 2006), after understanding their sex-driven arrival dynamics (Reinecke et al., 2002). However, host plant location mechanisms that drive female *C. zealandica* colonization patterns on plant foliage are still unknown. It has been suggested that the contrast that plant silhouettes produce in the sky at dusk contribute to plant host location in this species (Farrell & Wightman, 1972; Kelsey, 1951; Pottinger, 1968). At dusk, the relative amount of UV light present from the sky is extremely high compared with its amount from the ground (Mellor & Hamilton, 2003), producing the perfect contrast for insect orientation and navigation (Dacke et al., 2003; Mellor & Hamilton, 2003; Seliger et al., 1994). Also, moon brightness, starlight and milky way illumination might play a role in *C. zealandica* orientation (Kelsey, 1957, 1968; Radcliffe & Kain, 1971), as demonstrated before in the dung beetle *Scarabaeus satyrus* F. (Coleoptera: Scarabaeidae) (Dacke, Baird, Byrne, Scholtz, & Warrant, 2013). Further research might consider artificially creating the light characteristics present at dusk, as within a planetarium facility (Dacke et al., 2013). Despite the logistic drawbacks of releasing flying insects in such a building, an experiment revealing the mechanisms behind *C. zealandica* orientation at dusk might contribute to understand the colonization patterns of several adult Melolonthinae beetles that cause severe defoliation around the world.

6.1.3 Adult and larval distribution within vineyard blocks

Adult *C. zealandica* densities were higher at the edge of the vineyard block compared to its centre. This was probably due to a relict behaviour described in this species, where females land on plant foliage to release their sex pheromone to increase the likelihood of attracting males from surrounding areas (Merton, 1980). After landing on those, adults drop to the ground, where females lay eggs. This behaviour explained the higher larval densities found where higher numbers of adults were present. However, larval sampling was performed at only one site during 2014; therefore the larval pattern found in the Awatere Valley might be particular to that site, thus assumptions about larval distribution in other vineyards are difficult. Nevertheless, if the adult relict behaviour described before (Chapters 3 and 4) is not changed by other landscape characteristics (i.e., hills, mountains, rivers), it is expected that *C. zealandica* larval abundance will be higher close to adults' landing/mating sites. Thus, future work should explore *C. zealandica* larval distribution at several vineyards in the Marlborough area. The above-mentioned association between mating and oviposition sites has been also described in Brazil on *Phyllophaga cuyabana* Moser (Oliveira & García, 2003; Oliveira et al., 2007). Although this PhD work was not able to distinguish the proportion of adults coming from outside the vineyard from the ones from within, organically-based management in the under-vine areas, such as cultivation for weed control during September and early October, might reduce pupal populations (Kain & Atkinson, 1970) with potential impacts in further adult colonization and establishment dynamics within vineyard blocks. Adult densities found at the edge of the vineyards, and subsequently, their larval populations, are in agreement with the theory of females responding to plant silhouettes in the sky (Chapters 3 and 4), as vines might be the first plant silhouettes that females identify after starting their second flight phase (Chapter 3). In this sense, tall plants could contribute to divert them from the vines. It was demonstrated for *P. cuyabana*, that females prefer to land on tall plants compared to small ones (García et al., 2003). Further research should explore this avenue, as it might contribute to new strategies for sustainable adult *C. zealandica* pest control. Tall plants established in the hedgerows or headlands in the vineyard areas could attract females and therefore, males, reducing the number of adults landing on the vines, and subsequently their damage. In Chapter 5, it was demonstrated that a reduction in adult numbers landing on vines reduced the damage caused by this insect pest.

Nowadays, conventional winegrowers are reducing their pesticide loads, as their awareness of this current work grows, applying pesticides only at the edge of their vineyards. This approach has economic and environmental benefits.

6.1.4 Sustainable adult *C. zealandica* management in vineyards

Many good examples can be found in the literature where biodiversity has been managed to reduce pest numbers (Altieri, 1999; Gurr et al., 2017; Gurr et al., 2016; Gurr et al., 2012; Jonsson et al., 2008; Koohafkan, Altieri, & Gimenez, 2011; Kremen & Miles, 2012; Landis, Wratten, & Gurr, 2000; Parker et al., 2016; Reganold & Wachter, 2016; Snyder, 2009; Wratten et al., 2012). The mechanisms behind the effects of biodiversity on pest control are related to the increase in the numbers of natural enemies, such as generalist predators and parasitoids, in those agricultural landscapes, by enhancing shelter, nectar, alternative food, and pollen (Barnes, Wratten, & Sandhu, 2010; Gurr et al., 2017; Landis et al., 2000), but see (Tscharntke et al., 2016) when this approach fails. Generalist predators certainly do contribute to reducing pest populations (González-Chang et al., 2016; Symondson, Sunderland, & Greenstone, 2002). It has been previously suggested that *C. zealandica* larval and pupal generalist predators cannot contribute to effectively reduce their numbers (Cameron & Wigley, 1989; Jackson, 1990). This might be related to the study system, as those conclusions were made based on pasture ecosystems (Jackson, 1990). Also, the only know parasitoid for this species, *Proscissio cana* Hutton (Diptera: Tachinidae) (Thomas, 1963) attacks its larval host in spring and summer (Merton, 1980). Due the pastoral improvements in New Zealand during the last 40 years, *C. zealandica* larvae can easily increase their larval weight therefore, if no environmental constraints exist, such as low temperature or drought (Wightman, 1974a, 1974b), a one year-life cycle will be expressed, instead of the two-year life cycle necessary to provide the required hosts to its parasitoid during spring and summer (Merton, 1980; Perrot & Stockdill, 1973). This suggests that native New Zealand plants might have evolved with some root defences against *C. zealandica* larval feeding, allowing the parasitoid to evolve with its host. If an overall increase in biodiversity is desired within New Zealand vineyards as a strategy to reduce *C. zealandica* numbers, and promote several ecosystem services (Shields et al., 2016; Wratten et al., 2012), further research should focus on the relationship between native New Zealand plants, larval feeding and their effects on the natural enemy community of this endemic pest. Native plants might also contribute to divert females from landing on vines, through their silhouettes in the sky. Because such work requires more than the limited 3-year time of a PhD programme to produce a practical outcome, it is strongly recommended to explore this avenue as a strategy to produce a long-term sustainable management of this endemic scarab pest. In this sense, a control approach aimed at this insect's larval population control should consider the proportion of larvae within the vineyards and in their surrounding areas, as on-farm larval control alone might not ensure a reduction in adult defoliation, especially if adults are coming from surrounding vineyard areas. This framework also applies to the larval biological control produced by the bacterium *Serratia entomophila* (Grimont, Jackson, Ageron, & Noonan, 1988), as initially promising results found in pastures (Frew et al., 2016; Jackson, 2007) might not reduce adult defoliation in vineyards if regional larval population distribution is not considered.

For the reasons mentioned above, this PhD work explored naturally-based products that alter adult feeding and landing behaviour. In Chapter 5, it was demonstrated that hydrophobic particle films and diatomaceous earths reduced adult damage between 33 and 46%, depending on the studied year. Moreover, crushed mussel shells placed as mulch in the under-vine areas reduced adult damage by 73%, with an associated grape yield increase of 28%. This mulch has the property to reflect UV-B light, which affects plant physiology (Jansen & van den Noort, 2000; Osrečak et al., 2016) and insect orientation (Antignus, 2014; González-Chang et al., 2017). Although at dusk, UV-B levels are low (Théry, Pincebourde, & Feer, 2008), their relative intensity present in the sky compared to the ground might be enough to disrupt insect visual orientation (Mellor & Hamilton, 2003). Therefore, UV-B reflection from the ground covered by crushed mussel shells could "erase the vine silhouettes" from adult *C. zealandica* visual perception, confounding them with a portion of the sky. Further research should address the mechanisms behind mussel-shell effects on reducing adult hovering and landing activity on treated vines by measuring the light reflected from those crushed mussel shells at dusk. Also, different reflected mulches can be tested, as crushed mussel shells might not be available in all areas where vineyards are attacked by this endemic pest. The implications of alternative materials placed as mulch to deter Melolonthinae beetle landing behaviour on vines could have important effects on how this insect group is currently managed worldwide. Chapter 4 demonstrated that *C. zealandica* adults were concentrated at the edge of the vineyard blocks, and suggested that management strategies should focussed there. However, when mussel shells were placed in this area, adults landed further into the block. This result highlights another research avenue in which the distance from the edge towards the centre of the block which needs to be treated with mussel shells to avoid insects landing further into the vineyard, needs to be addressed. That final piece of knowledge might complete the puzzle related to adult *C. zealandica* sustainable management in vineyards, allowing the creation of a protocol that can easily be applied by New Zealand winegrowers, especially for organic and biodynamic ones.

In conclusion, through studying aspects of *C. zealandica* adult behaviour, this PhD work contributed with an ecologically-sound pest management approach, which is in tune with New Zealand wine industry needs, and also with a scientific "way of thinking" that conceives the sustainable management of insects by evaluating aspects of their behaviour, instead of just applying pesticides. One of the contributions of this work was demonstrating that *C. zealandica* females arrive before males on vines. After females land, male distribution is likely affected by female pheromone release. Due to this sex-driven colonization dynamic, adults were concentrated at the edge of the studied vineyards which partly contributed to higher larval densities found at the same area. Higher pest aggregation at the edge of the crop suggests that control strategies should focus that area, reducing the overall pesticide load in that particular vineyard block. In this work, all the alternatives to

synthetic pesticides tested produced a significant reduction in vine damage (from 33 to 73%) when applied at the edge of the vineyard block. This result highlights their feasibility for being used in commercial vineyards as alternatives to current pesticide applications. Although mussel shells applied in the under-vine areas produced a dramatic reduction in vine damage (73%), and subsequently a 28% increase in grape yield, their application only at the edge of the block changed the colonisation pattern of flying adults. Certainly, this change in adults' behaviour needs further investigation. Because this work was generated by the need that New Zealand winegrowers had to manage this pest without using pesticides, the techniques evaluated in the current work are gradually being applied by Marlborough winegrowers. However, it is expected that the outcomes of this work help winegrowers throughout other New Zealand areas, and even in some parts of Europe, Asia and South America, where melolonthid species heavily defoliate their orchards. In New Zealand, industry motivations for contributing with funding to the research presented here arise partially because of pesticides' adverse effects on the environment and human health, but also due to cultural and spiritual values present in parts of New Zealand society. At a more strategic level this work highlighted the urgent need for sustainable agricultural practices to meet food security for an increasing human population without having undesired environmental and social effects. It is well known that agriculture will face several challenges in the following decades, such as climate change, human overpopulation and land degradation; however, there is no scientific consensus on how they will be faced. In this sense, scientific research looking at ecologically-sound management strategies that enhance several ecosystem services at farm level will certainly contribute to advance into the mitigation of those challenges.

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